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MALARIAL PARASITOLOGY.*

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INTRODUCTION.

Although the chief interest in the investigation of malaria centres at present in the study of experimental inoculations, of the natural mode of infection, and of the extracorporeal forms of the parasite, much yet remains to be learned about the morphology of the organism in its several varieties. The minute study of the morphology of the parasite has furnished far more cogent evidence of the existence of several species of the plasmodium of human malarial fever than has that of the clinical manifestations of the disease. Yet even the tantamount question of the unity or plurality of species is still far from satisfactory solution. Moreover, many details in the biology of the plasmodium of malaria, including the development of the crescentic bodies, whose significance has been partly determined, still remain

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obscure and require further observations on the occurrence and behavior of the parasite in the blood.

My observations on a considerable number of cases of Cuban malarial fever, seen at Montauk during the summer of 1898, although pursued primarily for the purpose of immediate diagnosis, bear on the topics mentioned. The conclusions reached at that time and more especially the deductions drawn from a more leisurely review of the material then obtained it is proposed to consider in the present article.

I. TECHNICS.

Experience with the technics of blood examinations in malaria has led me to restrict the use of fresh blood to the study of various vital phenomena in the parasite, such as amœboid movement, vibratory motion of pigment, and exflagellation. When parasites are scanty their discovery is so much more certain and rapid in stained dry specimens that a negative result with fresh blood invariably requires verification by search through a dry specimen, stained preferably by Nocht's method. Moreover, exclusive reliance upon fresh blood not only leads constantly to errors in diagnosis by beginners, but also has been the cause of many erroneous conceptions held in the past by experts.

The study of flagellate bodies may be conducted in fresh specimens prepared in the ordinary way, but placed by preference on a warm stage. The addition of a little water may facilitate the escape of the parasite from the cell and the formation of flagella. The successful action of water has been obtained by several expedients. Marshall added about an equal quantity of water to a small drop of blood containing many crescents and saw the almost immediate change of crescents to spheroidal bodies, followed by flagellation. Manson[1] recommends that the blood under the cover-glass be kept moist by exposure to steam exhaled from a hot, moist sponge. After a few minutes the cover may be carefully removed, the specimen dried, and the flagellate body stained.

Bignami studied in detail the flagellate bodies in specimens rather thickly spread, and prevented from drying in a warm moist chamber, while exflagellation occurred, with subsequent drying and staining. I find that a moist chamber may be conveniently secured in a Petri dish with tightly fitting vaselined cover. Wet blotting paper placed in the dish furnishes the necessary moisture. Specimens spread on slides or

[1] *Lancet*, 1896, ii, p. 1715.

covers may be kept moist for 10 to 20 minutes in such dishes, and flagellation proceeds with moderate rapidity.

Staining Methods.

1. *Eosin and Methylene Blue.*—For all ordinary purposes staining by eosin and methylene blue may be generally recommended, and was largely employed in the present cases. The solutions required are: (*a*) a saturated alcoholic solution of alcoholic eosin diluted with an equal quantity of 95 per cent alcohol, and (*b*) a saturated watery solution of Ehrlich's rectified methylene blue at least one week old.

A light staining by eosin, such as is given by the diluted solution of eosin, is essential for the clear demonstration of the parasite by methylene blue, and in specimens containing only the small signet-ring forms, heavy staining by eosin may almost entirely prevent the subsequent action of methylene blue, and these minute parasites may thus be overlooked.

2. Methylene blue fails to stain the young ring forms, especially those of the tertian type, as clearly as is desirable, and more powerful basic staining fluids may well be employed for this purpose. Nocht's method may be recommended over any other, as it facilitates the identification of the small ring by means of a large densely stained nucleus, but when this method cannot be employed, one may resort to the method modified by Futcher and Lazear from the suggestions of Benario and Marchoux, as follows: Fix the specimens five minutes in 95 per cent alcohol, 100 cc., to which has been added 1 cc. of formalin. Stain one to three minutes in the following mixture: sat. alc. sol. thionin, 20 cc., 20 per cent carbolic acid, 100 cc. The fixing solution must be used fresh, and the staining fluid must be at least one week old. The rings are then densely stained and the specimens do not fade.

3. The sharpest demonstration of minute ring-shaped parasites was obtained in the present cases by staining one hour in diluted Gage's hæmatoxylin, before treatment by eosin and methylene blue. Hæmatoxylin stains the nucleus of the ring and makes the body of the parasite blacker after methylene blue. Such specimens are specially suitable for photography.

4. *The Nocht-Romanowsky Method.*—In 1890 Romanowsky published a method of staining the malarial parasite, which apparently demonstrated certain nuclear structures very imperfectly shown by other methods. The original description of this method directed that the specimens, fixed in equal parts of alcohol and ether, or by heat at

110° C., be placed for two to three hours in a fresh mixture made by adding concentrated (1 per cent) solution of methylene blue, 1 part, to 1 per cent watery solution of aqueous eosin, 2 parts. The red corpuscles were stained light pink, and the body of the parasite blue, while the chromatin particles of the nucleus appeared deep red.

The sketches accompanying Romanowsky's article were absolutely convincing that the new method was a most valuable addition to existing technical procedures, but it was soon found that the results obtainable from this method were extremely uncertain. We now know that only those who happened to secure particular specimens of methylene blue obtained successful results with Romanowsky's stain, and that the later highly purified products of the aniline-dye factories seldom contained the effective agent which united with the chromatin granules. Romanowsky believed that by the mixture of methylene blue and eosin a third compound was formed which stained the chromatin. Attention was not fully drawn to this probable explanation of the difficulty until Ziemann published the results of his experience with various specimens of methylene blue. After numerous attempts to stain by Romanowsky's method, following the exact directions, he sums up his results in the few words: "glückte kein Präparat." After a long series of experiments he found that a successful result depended upon the variety of methylene blue, the age of the solution, the quantity of eosin used, and the length of exposure. He secured most uniform results from the use of 1 per cent aqueous solution of methylene blue (med. puriss., Höchst) or Koch's methylene blue (Grübler), or Ehrlich's rectified methylene blue (Grübler). He found no difference in the effects of various specimens of eosin, but used principally a 1 per cent aqueous solution of watery eosin (Höchst). The proportions of these solutions required varied from 1 to 7 parts of eosin added to 1 part of methylene blue, and the time of staining from 15 to 40 minutes. The proportions of the dyes and the time of staining had to be determined for each specimen of dye. The older the solution of methylene blue, the less eosin was required in the mixture.

Gautier, who was very successful with Romanowsky's method, had the best results by using specimens of methylene blue marked C and BGN, and of eosin marked A, of the Baden Anilin factory. He found considerable difference in the results obtained with different specimens of eosin. Many other observers had a variable experience with the method, but it was generally agreed that a successful result was very uncertain and depended on factors little understood. I had few successful results in

following Romanowsky's method and just as many failures with Ziemann's procedures.

One turns with relief, therefore, to find that a modification recently suggested by Nocht furnishes a method which is invariably successful, without much dependence on the quality of dyes used, or the length of staining. Nocht's modification consists in the addition of a few drops of neutralized Unna's polychrome methylene blue (Grübler) to the 1 per cent solution of ordinary methylene blue. The usual specimen of polychrome methylene blue is distinctly alkaline and to be rendered effectual for the present purpose Nocht found that it required neutralization, preferably by acetic acid. This may be done by adding drop by drop of dilute 2 to 3 per cent acetic acid till the commercial fluid polychrome blue no longer turns red litmus-paper blue above the zone coming into immediate contact with the dye. I have never failed to secure a good result by the following procedure:

(1) To 1 oz. of polychrome methylene blue (Grübler) add 5 drops of 3 per cent solution of acetic acid (U. S. P., 33 per cent).

(2) Make a saturated (1 per cent) watery solution of methylene blue, preferably Ehrlich's rect. (Grübler), or Koch's, dissolving the dye by gentle heat. This solution improves with age, and should be at least one week old.

(3) Make a 1 per cent solution in water of Grübler's aqueous eosin.

The mixture is prepared as follows:

To 10 cc. of water add 4 drops of the eosin solution, 6 drops of neutralized polychrome blue, and 2 drops of 1 per cent methylene blue, mixing well. The specimens, fixed in alcohol or by heat, are immersed for two hours, specimen side down, and will not overstain in 24 hours. The density of the blue stain may be varied to suit individual preferences. The above proportions need not be rigidly followed, but the polychrome solution should be accurately neutralized.

Nocht later reports that the two solutions of methylene blue may be replaced by a 1 per cent solution of Ehrlich's rect. methylene blue, alkalinized by a few drops of $\frac{1}{2}$ per cent of sodium hydrate, and left a few days in a thermostat at 50° C. This is the ordinary laboratory method of improvising polychrome methylene blue. With this preparation the procedure is as follows: To 2 cc. of water add 2 to 3 drops of 1 per cent watery eosin, and drop by drop of the alkalinized methylene blue till the original red color of the eosin has almost disappeared. In this fluid specimens stain in 5 to 10 minutes. I have had little success with this method.

The rationale of the Nocht-Romanowsky method is not yet fully un-

derstood, but it appears most probable that a staining agent which unites selectively with chromatin exists ready-formed in polychrome methylene blue, and may be developed in specimens of methylene blue in various ways, among which is slow digestion with an alkali and heat. Nocht refers to this agent as "red from methylene blue." It is not the commercial methylene red, but may be extracted from polychrome methylene blue, etc., by chloroform (Nocht), and it is a reasonable expectation that it can be put on the market in pure form.

Nocht's method furnishes so much information regarding the minute structure of the parasite and renders the identification of the parasite so complete and positive that it must be recommended above all others. Moreover, it has a large field of application in the study of nuclear structures in various other microorganisms.

Goldhorn (N. Y. Path. Soc., Feb. 13, 1901) has recently succeeded in digesting polychrome methylene blue with lithium carbonate, neutralizing with acetic acid, so as to develop in it a large proportion of the red-staining principle. His method is as follows: Fix the preparation, which must be fresh, by immersion in pure methyl alcohol for 15 seconds. Wash in water and stain 7 to 30 seconds in 0.1 per cent aqueous solution of eosin. Wash and stain in digested polychrome blue 30 to 60 seconds.

Besides rapidly staining the chromatin of the parasite, Goldhorn's fluid demonstrates most exquisitely the early appearance of extreme granular degeneration of the infected red cell, in which respect it is a distinct improvement over any stain yet devised. The fluid can be obtained from various dealers in New York City.

II. GENERAL MORPHOLOGY OF PARASITES.

The Tertian Parasite.

The *youngest form* of the tertian parasite seen in the red cell is identical in appearance with the spore of the parent rosette. It is a compact spheroidal, or slightly oval, or irregular body, about 2 μ in diameter. It shows an outer rim of basiphilic protoplasm enclosing a single large nuclear body, which is achromatic to methylene blue but stains readily in hæmatoxylin or by Nocht's method, and which is usually enclosed or accompanied by a clear achromatic spot, termed by Gautier "the milky zone" (Plate XXIX, Fig. 1). In the fresh condition these bodies are noticeably refractive, especially the nucleus,

change their position but rarely their shape, and are never pigmented. From the earliest period of infection the red corpuscle is often swollen, and exhibits advanced granular degeneration.

Within a few hours after the chill the parasite is usually found to have assumed a somewhat characteristic ring shape, which it commonly maintains in some definite form up to the presegmenting stage (Plate XXIX, Figs. 2-11). These ring-shaped bodies measure from 3 to 4μ in diameter, and the regular ring form is retained, without marked increase in bulk at any point, for 6 to 8 hours. Sometimes the ring is elongated, one arm reaching across the cell, while a thin bow persists. Occasionally the ring appears to unfold, and the parasite stretches clear across the swollen cell, with the nucleus at one end. The tertian ring is rarely as geometrical or delicate as is the æstivo-autumnal signet ring. The development of pigment was inconstant in the present cases, some large rings failing to show pigment, but usually one or more fine grains were to be seen in the medium-sized and smaller forms. The ring always encloses a considerable mass of hæmoglobin. The nuclear body of the tertian ring is its most characteristic feature, appearing as a rather large, achromatic, highly refractive body, after methylene blue, but staining intensely with hæmatoxylin and by Nocht's method.

Significance of the Ring Form.—In regard to the formation and significance of the ring opinions are at variance. Most of the Italian writers hold that the ring form is not really a ring, it being bridged across by a transparent and vesicular nucleus. There are many considerations favoring this view, especially the usual appearance of the parasite in the fresh condition, and the fact that the chromatin usually lies within the ring, eventually filling it. On the other hand, Mannaberg and Ziemann claim that this body is a true ring, formed by the thinning of the central portion of the body, and state that they have seen the ring develop in this manner in fresh blood. From the examination of the rings themselves I have been unable to convince myself as to which view is correct, but there are some early forms of the parasite which strongly indicate that the ring does not represent a vesicular nucleus. In one such form the ring is unfolded

and the nucleus lies naked in the haemoglobin. Elongated forms of the young parasite are often seen in which the ring is absent and the nuclear body lies bare at one end. These forms vividly recall the appearance of *Amœba coli*, in which the nucleus remains at the hinder end during active movements of progression. Further, it is difficult to associate the relatively huge size of the ring with any nuclear structure, as this would require the young malarial parasite to have a nucleus which is larger than that of the adult *Amœba coli*. Again, secondary rings sometimes form from the union of pseudopodia, and these are identical in appearance with the primary ring but lack the chromatin granules (Plate XXIX, Figs. 6 and 8).

In specimens stained by Nocht's method the chromatin is usually found within the ring, sometimes lying in an isolated position in the centre, but very often the chromatin is found *outside* of the ring, connected by a very fine thread of protoplasm. If the ring represents a vesicular nucleus, we have here the anomaly of a complete separation of chromatin from the vesicular portion of the nucleus, which is opposed to some rigid histological principles. Even more frequently the chromatin is found to be enclosed by bluish staining protoplasm which shuts it off entirely from the ring (Plate XXIX, Figs. 7 and 8).

From various biological studies it appears that the nuclei of the protozoa are usually widely different from those of the metazoa: that many protozoa do not have a vesicular nucleus, with cell membrane, linin, nucleolus, etc., but possess the so-called "distributed nucleus" composed of a number of granules lying free in the body of the parasite. The study of the malarial parasite by Nocht's method indicates that the nucleus of this protozoon is of the *distributed type*, which does not exhibit a vesicular structure nor possess a nuclear membrane.[2] On these grounds I am inclined to agree with Mannaberg and others who hold that the form in question is a true ring—a form usually, but not necessarily, assumed by the parasite—and does not represent a vesicular nucleus.

Comparison of the Tertian and Æstivo-autumnal Rings.—From the study of the ring-shaped tertian parasite and the æstivo-autumnal

[2] See Calkins on "Protozoan Nuclei" in Annals N. Y. Acad. Sciences, 1898, xi, Part iii.

signet-ring forms, in typical cases of these infections, it appears to me that these parasites, with very rare exceptions, can be fully distinguished from each other, even in this early stage, by the following peculiarities:

(1) The nuclear body and chromatin mass of the young tertian parasite is achromatic to methylene blue, which densely stains the nucleus of the æstivo-autumnal organism. I have been unable to find in the literature any specific reference to this peculiarity as a diagnostic point, but it may be readily verified by comparing specimens of the two parasites stained by methylene blue and by Nocht's method. The dense staining of a nuclear body in the young æstivo-autumnal parasite has often been noted, and in 1894 Okintschitz mentioned the fact that the nucleus of the young tertian parasite fails to stain by methylene blue.

(2) The tertian ring is usually coarse and irregular, but the æstivo-autumnal ring is geometrically circular, more delicate, with an extremely fine bow, and usually with a typical signet-like swelling.

(3) One or two grains of pigment are almost invariably found in the early tertian ring, but are, with nearly equal constancy, absent from the æstivo-autumnal signet-ring.

(4) My specimens confirm the statement of Gautier that the tertian ring is usually pigmented before the chromatin becomes subdivided, while the chromatin of the æstivo-autumnal ring is always subdivided before the appearance of pigment. In some cases, however, the chromatin of the tertian ring divides before the appearance of pigment.

(5) The infected cell is usually swollen from the moment of infection by the tertian spore, and commonly shrunken when harboring the æstivo-autumnal ring.

All of these characters are usually apparent in ordinary specimens, but naturally are most distinct in flatly spread and rapidly dried corpuscles. I have encountered no exception to these rules in cases of infection by the tertian parasite in New York, and cases of æstivo-autumnal infection from Cuba. In many of the irregular relapses in cases showing tertian organisms encountered among volunteer

soldiers during the winter of 1898-99, single ring-shaped parasites not admitting of positive identification have frequently been seen. The significance of this observation will be considered later (see section on Plurality of Species of Malarial Parasites, p. 490).

After a period of 6 or 8 hours the tertian ring is usually found to have developed an outgrowth which is actively amœboid in the fresh condition and appears in stained specimens as a tongue-like protrusion or turban shaped mass attached to one segment of the ring (Plate XXIX, Figs. 4 and 5). The nuclear body meanwhile increases slightly in size, projecting into the ring, and the chromatin divides into several large granules.

At this period occurs the greatest amœboid activity of the parasite, and in some severe tertian infections the parasite may be found fixed in the height of its excursions, when it presents in stained specimens the peculiar appearance depicted in Plate XXIX, Figs. 9 and 10. Here the ring is unfolded and the body of the parasite is strung out into a number of slender threads with nodal thickenings. At times the number and delicacy of the threads greatly exceed those seen in the sketch, and in some red cells the parasite may be found distributed in a series of fine granules between which the connecting threads are with difficulty distinguished. These latter are probably to be classed with "quinine forms."

A close inspection of corpuscles harboring such parasites often discloses the presence in one corpuscle of two distinct nuclear bodies, indicating the co-existence of two parasites. Frequently in one corpuscle are found twin parasites, entirely separate one from the other, each of which shows a tendency to develop the long threads (Plate XXIX, Fig. 9). When, however, the threads are numerous and very thin it is usually impossible to find any break in their continuity, while in many instances the two parasites are distinctly united. The significance of these two forms will be considered later (p. 475 et seq.).

During the second quarter of the cycle the body and the nucleus of the parasite develop rapidly in size, amœboid motion and amœboid figures gradually diminish, and pigment is abundantly deposited in the form of fine dark brown or yellowish grains showing in the fresh

state active vibratory motion. The infected red cell continues to increase slightly in size, its hæmoglobin is progressively diminished, and granular degeneration is extreme. Depending upon the character of amœboid activity the variety of figures seen during this period is very great. Eventually, toward the end of 24 hours or possibly somewhat later, the parasite occupies three-quarters of the swollen cell, in the form of a spheroidal or elliptical, homogeneous body, the outer portion of which contains most of the pigment and is rather more deeply stainable than the zone immediately surrounding the nucleus (Plate XXIX, Figs. 12 and 13). The nucleus gradually increases in size, growing into the ring. It no longer has the appearance of a small highly refractive achromatic spot (after methylene blue), but takes a light bluish tinge with 1 per cent methylene blue, and stains less deeply than before with hæmatoxylin. At the end of this period the nucleus completely fills the ring, stains rather distinctly with methylene blue and sometimes exhibits a delicate bluish network.

By Nocht's method the changes in the nucleus are found to consist in the gradual subdivision of the chromatin granules, which finally become rather numerous, of minute size, and more difficult to stain. Usually these chromatin granules lie on the inner circumference of the bow of the ring, projecting within the ring, and partly surrounded by a "milky" unstained zone. This milky zone is often absent in young parasites in cells thinly spread and rapidly dried, but in older parasites it is always present.

Various other positions may be assumed by the chromatin mass, as follows: a subdivision of the granules into two distinct groups, separated by a strand of bluish stained protoplasm; an eccentric position entirely apart from the ring; a position midway between two rings formed in the same parasite; a position in the centre of the ring entirely apart from any bluish protoplasm; a circular arrangement about the periphery of the ring. Sometimes the smaller granules are grouped about a central larger granule, as has been noted in other protozoa whose nuclei are of the "intermediate type" (*Microglena*, *Euglena*).

The third quarter of the cycle is occupied by the continued growth of the parasite in the form of a large homogeneous richly pigmented body, which finally occupies at least four-fifths of the swollen red corpuscle, and by certain nuclear changes which it is difficult to follow in specimens stained by methylene blue or hæmatoxylin, but which are fully demonstrated by Nocht's method.

The exact limits within which the parasite may be termed "full grown" can be sharply fixed only with great difficulty, but there appears to be a period of at least 12 hours, during which there is little change in the structure of the organism and during which the body stains homogeneously and the nucleus occupies the entire ring. This period may be placed between the 24th and 40th hour of the cycle. A portion of it is occupied by nuclear changes belonging to the reproductive phase of the parasite's development.

After the appearance of a faint intranuclear network most authorities agree that the nucleus largely disappears, so far as can be determined in specimens stained by methylene blue or hæmatoxylin, and that it is next seen in the form of highly refractive achromatic spots in the meshes of the reticulated presegmenting body (methylene blue), and these again stain deeply with hæmatoxylin.

Nocht's method, however, fully demonstrates the nuclear changes which occur in the full-grown parasite. A considerable area, usually the entire original ring, is now occupied by a "milky" or slightly bluish staining substance in which lie a considerable number of very fine chromatin granules. These granules are usually difficult to stain, and being of very minute size they are difficult to see. This fact has led Ziemann and Gautier to admit the possibility that the chromatin may actually disappear at one stage of the development, especially since they have found some large parasites in which no chromatin was demonstrable. In my specimens there were a very few large tertian parasites in which no chromatin granules appeared, but these were not more numerous than younger forms which were also devoid of chromatin and must therefore be regarded as sterile. I therefore interpret the larger forms devoid of chromatin as sterile forms, and cannot accept the view that the chromatin entirely dis-

appears at any stage of the fertile parasite, a view which is at variance with biological principles.

After the subdivision of the chromatin has reached a limit the next change, observed in a considerable number of parasites, appears to consist in the extension of a portion of the milky substance and its chromatin granules into the body of the parasite (Plate XXIX, Fig. 13.) At the same time the granules of chromatin increase in number. Other forms may be seen in which the "milky substance" and chromatin granules occupy an elongated space within the body of the parasite, and in such cases the beginning concentration of pigment and deeper stain of the parasite indicate the presence of the presegmenting stage (Plate XXIX, Fig. 14).

Presegmenting Bodies.—In specimens stained by methylene blue the first demonstrable indications of the division of the parasite are seen in *deeper staining capacity* and *tendency toward reticulation* which appear throughout the whole or in a part of the body of the parasite. Occasionally these changes may be noted in one-half the parasite while the other half retains the homogeneous appearance of the "full-grown" organism. Usually the process is found to have affected the entire organism, giving the very characteristic forms sketched in Plate XXIX, Figs. 15 and 16. In the presegmenting bodies the pigment is gathered in a reduced number of coarse grains, which lie in the body of the parasite in a position determined by that of the new multiple nuclei.

These bodies were first described by Golgi in fresh blood and properly interpreted as belonging to the process of segmentation. Later they were described by Marchiafava and Celli[1] as vacuolated parasites, the highly refractive nuclear bodies appearing in the fresh condition very much like vacuoles. Still later, Celli and Guarnieri sketched them from specimens stained in the fresh condition, regarding some as showing partial segmentation, others as vacuolated parasites, although they accurately described the appearance of the nuclear particles invariably found within these "vacuoles," while still others they supposed to be groups of confluent parasites, *i. e.* true plasmodia. Mannaberg's descriptions (1899), referring only to fresh blood, do not include these bodies, nor

[1] *Atti della R. Accad. med. di Roma*, 1887, 2. S., iii, p. 257.

have they found a distinct place in his plates, although some of the figures in his Plate IV indicate that they have not escaped his observation.

Thayer and Hewetson,[1] in their careful study of the parasite in fresh blood, designate as presegmenting bodies the parasites with collected masses of pigment (*corpi con blocchetto* of the Italian writers).

Laveran[2] refers to the similarity in appearance between a nuclear body and a vacuole, but he neither describes nor depicts the presegmenting reticulated parasite.

Ziemann[3] describes the presegmenting bodies as they appear after his or Romanowsky's staining methods, but the plates would not enable one unfamiliar with the subject to identify these forms in specimens stained by eosin and methylene blue.

The reticulated presegmenting tertian parasites were seen in every case of the present series examined within the 6 or 8 hours preceding the chill, and often in belated parasites shortly after the chill. Many transitional stages between the homogeneous adult parasite and the perfect rosette may be seen in rich infections. They are well demonstrated by eosin and methylene blue, especially as regards the increasing density of stain and the reticulation. After hæmatoxylin the multiple nuclei stain deeply by methylene blue.

By Nocht's method a series of interesting nuclear changes may be followed in the presegmenting forms. After the mass of enlarging chromatin granules and milky substance has flowed out into the elongated form described above, the chromatin granules leave the central clear space and make their way in groups out into the body of the parasite. Various stages of this process may be followed in specimens taken at suitable periods, and some observed phases are seen in Plate XXIX, Figs. 13-16. Considerable difference in the numbers of such groups may be noted in different cases. Usually a large number of ill-defined groups are seen, before the central mass of granules is exhausted (Plate XXX, Fig. 14). In one specimen the compact nuclei of the young spores appeared to form in one segment of the parasite before the main mass of granules had become exhausted.

[1] *The Johns Hopkins Hospital Reports*, 1895, v, p. 3.

[2] Traité du paludisme. Paris, 1898, p. 62.

[3] Ueber Malaria- und andere Blutparasiten. Jena, 1898.

Each of the groups appears always to be surrounded by a milky zone, and the mass of granules is often of a peculiar triangular form (Plate XXIX, Fig. 12). During these changes the pigment granules increase in size, diminish in number, and are distributed in the meshes of the now distinctly reticulated body of the parasite.

Tertian rosettes (Plate XXIX, Fig. 17) are usually seen in the circulation three or four hours before the chill, most abundantly just before the chill, and a few are often to be found for one hour or more after the chill. These limits may occasionally be much wider, as Marchiafava and Celli have seen rosettes 2 to 6 hours before the chill, and 6 to 7 hours thereafter; and indeed, when the different broods of parasites are not very distinct, there is no reason why occasional rosettes should not be found at any period of the main cycle.

Of the three types of sporulation described by Golgi, the second, according to which the entire parasite is divided into spores, leaving nothing but pigment, is undoubtedly the usual process. As regards Golgi's first type, in which only the peripheral portion of the parasite divides, leaving a distinct central globular pigmented body, most stained specimens fail to show convincing evidence that the physiological process of segmentation may be subject to such an important modification, nor does it appear in recent literature that the existence of this variety of segmentation has been fully verified. In a few specimens from patients taking quinine, I have seen rare segmenting bodies which resemble those described as above by Golgi, but never in fresh cases.

Golgi's third type of "partial segmentation," together with the "lateral circumscribed sporulation" of Celli and Guarnieri, may frequently be seen in rich tertian infections in fresh blood, but according to the evidence of stained specimens this must be classed with the presegmenting forms.

The tertian rosette is usually distinguished by its large size and considerable number of spores—fifteen to twenty. In the present cases it did not appear, however, that the identification of the tertian rosette could always be based upon the number of spores. Marchiafava and Bignami have described tertian rosettes with 40 to 50 spores.

With the smaller number of spores the rosette was usually much larger than either the quartan or the æstivo-autumnal body.

The nuclear changes demonstrated by Nocht's method in the tertian rosette consist principally in the gradual fusion of the new-formed groups of chromatin granules into single compact globules, which are partly surrounded by "milky zones." While the rosette is still compact the vesicular shape of the spore is distinct. The outer segment of the ring is usually thickened, the nucleus tends to lie near the inner pole, and between the nucleus and outer segment is a small "milky zone." The pigment is usually collected into a central block or mass of granules, but may be found variously scattered among the spores, or along the periphery of the rosette.

The Quartan Parasite.

The earliest form of the quartan parasite (Plate XXXI, Fig. 1), as seen in the stained red cell, is practically indistinguishable from that of the tertian organism, but its true character may usually be suspected from the slightly shrunken appearance of the infected cell. In fresh specimens the higher refractive quality of this parasite is often, however, sufficiently characteristic for its identification. After a very slight increase in size the quartan parasite becomes rather easy to distinguish in both fresh and stained specimens, for it usually remains smaller, more compact, and is more richly and coarsely pigmented than the tertian organism. As with the latter, the nuclear body is found projecting into the ring (Plate XXXI, Figs. 1-4). In fresh specimens at this period, the higher refractive qualities and slower amœboid motion are additional diagnostic characters.

The growth of the quartan ring is very similar in all important respects to that of the tertian, while its distinguishing features, especially the abundance of coarse pigment grains, are uniformly retained (Plate XXXI, Figs. 5-8).

During the presegmenting stage the characters of the quartan parasite are markedly different from those of the tertian. On account of the slower progress of sporulation, and from the greater tendency of the quartan parasite to complete its cycle in the general circulation, quartan presegmenting bodies are relatively much more numerous

in the stained specimens than are the similar forms of the tertian organism. In some specimens taken several hours before the chill the majority of organisms found may present the markedly reticulated structure indicative of approaching division. The multiple nuclei are less numerous, and the pigment is more abundant, and is often found in irregular, partly radiating rows. The coarsely reticulated, relatively small, and richly pigmented bodies lying in markedly shrunken cells are very characteristic and not readily confused with any other form of malarial parasite commonly found in the peripheral blood (Plate XXXI, Figs. 10-12). In some severe æstivo-autumnal infections, showing many parasites of all stages in the peripheral blood, somewhat similar spheroidal or presegmenting forms of the same general appearance may be found in considerable numbers, but as will be seen by reference to Plate XXX, the character of the pigment in the æstivo-autumnal parasite is very different. Such cases are very rare, and readily recognized on clinical grounds, being almost invariably of the pernicious type.

The quartan segmenting bodies are relatively more abundant in the peripheral circulation than are rosettes of any other variety of malarial parasite, and are easily identified by the small number (6 to 12) and comparatively large size and geometrical arrangement of the spores (Plate XXXI, Fig. 14).

The Æstivo-Autumnal Parasite.

The following description applies to a group of organisms which, according to the Italian school, comprises two or three varieties of malarial parasites. Waiving for the present the question of plurality of species, the entire group will be described as one, and the grounds alleged for their separation will be considered later. The present description rests upon the examination of some 260 cases of æstivo-autumnal infection occurring in U. S. soldiers who had shortly before arrived at Montauk Point from Cuba, and on a smaller number of cases seen in New York during the past few years. The conclusions drawn from the original examinations have been verified or modified by more careful study of these specimens during the past winter and also by the microscopical study of the tissues of a number of cases coming to autopsy.

The *earliest form* of the æstivo-autumnal parasite seen in the red cells, in the present cases, was very similar to that of the tertian and quartan parasites, but was slightly smaller than either, and was often distinguishable from the tertian by the shrinkage of the cell, and from the quartan by its distinctly smaller dimensions (Plate XXX, Figs. 1 and 2). In fresh specimens the young amœboid body usually showed a low refractive index as compared with the tertian and quartan parasites. It was never pigmented. Associated with the intracellular spores there were frequently seen in the plasma small spheroidal bodies, exhibiting an active rolling motion and occasional blunt projecting points (pseudopodia?), which, on becoming arrested by contact with red cells, were found to be indistinguishable from the intracellular bodies. Although seldom identified in stained specimens, it appears probable that these bodies were young extracellular forms of the parasite. The positive identification of these extra-cellular bodies, however, appears to be a very hazardous undertaking (cf. Ziemann, p. 49). In dried specimens stained by Nocht's method, however, the young extra-cellular parasite may be positively identified from the presence of a characteristic mass of chromatin. In my specimens such extra-cellular bodies were rarely encountered.

At a very early period of its development the æstivo-autumnal parasite in the present cases assumed a very characteristic *ring shape*. Many of these rings early developed a thickening of one segment, and to these bodies of various sizes the term "signet-ring" very aptly applies (Plate XXX, Figs. 5-7). It was noted that in some cases the rings failed to exhibit this thickening, but remained of a *uniform but very fine caliber throughout* (Plate XXX, Fig. 4). The period during which the rings retained this uniform caliber was not determined, but bodies of this type were seen measuring at least 3 μ in diameter. They nearly always presented two nuclear bodies, lying at opposite poles or close together. Occasionally such rings were found to have unfolded and to be stretched like a thread across the cell, the nuclei appearing at inconstant intervals. In other cases no rings of this type were seen, all showing the thickening of the signet and a single nuclear body. In the majority of cases rings of both types were associated in variable numbers.

No connection was demonstrated between the clinical features of these cases and the occurrence of these two types of rings, as both were found in cases showing intermittent tertian paroxysms, quotidian paroxysms, remittent fever of seven days' duration, and irregular fever for longer periods.

Multiple infection with the young rings was very common in the red cells of most cases of the present series and, as a rule, its occurrence was proportionate to the severity of the disease. In the peripheral blood three parasites were often found in the same red cell, occasionally as many as four; while in smears of the marrow of a fatal case infection of one red cell with four rings was common, five parasites were occasionally seen in the same cell, and one slightly swollen red cell was encountered containing seven well-formed rings (Plate XXX, Fig. 2). These observations accord with those of Ziemann (op. cit., p. 49), who found often three and four parasites, and once as many as five, in one red cell.

It appears in the descriptions of *Haemamoeba immaculata*, which is said to sporulate without producing pigment, that most of the rosettes contain comparatively few spores, averaging from 6 to 10 (Marchiafava, Bignami, Ziemann, Marchoux, Grassi and Feletti). The close resemblance to a non-pigmented rosette presented by some of these red cells harboring 5 or 6 young parasites was very striking. In my specimens (Plate XXX, Fig. 2) there could be no doubt as to the proper interpretation of the appearances.

Multiple infection of the red cell appears in rather rare instances to lead to the development of a peculiar form of the young æstivo-autumnal parasite on which Mannaberg bases his unique theory of the development of crescents. This body consists in the apparent union of two rings by a fusion of their nuclear bodies (Plate XXX, Fig. 3). Mannaberg depicts all transitional forms between these bodies and the fully developed crescent. Many examples of these double rings were encountered, but the various transitional forms from young double rings to crescents, shown in Mannaberg's plates, were not seen in the present cases.

The signet-ring forms frequently reached a diameter of 4 μ, while

still retaining the peculiar thickening of one segment and a very distinct nuclear body staining with methylene blue and surrounded by a narrow achromatic zone. Plate XXX, Figs. 6 and 7). Beyond this size, when persisting in the peripheral blood, the growth of the parasite produced an irregular body in which the outline of the ring became more or less obscure. The exact periods required in the development of these rings were not definitely determined, but in six cases taking quinine typical signet-ring forms were seen in the peripheral blood 60 to 72 hours after the beginning of the paroxysm. It seems probable, however, that these were belated individuals belonging to a somewhat scattered brood of which the majority either had retired to internal capillaries or had been destroyed by quinine. Yet in some cases in which the blood changes were followed at intervals of 6 to 12 hours, the increase in the size of the rings proved to be surprisingly slow, and the impression was obtained from these cases that the full development of the signet ring usually requires at least 24 hours and sometimes longer.

In the majority of cases the ring forms seen in the peripheral blood failed to show any trace of pigment, especially in patients showing distinctly intermittent quotidian or tertian paroxysms. In a considerable number of instances, however, especially in very severe and fatal infections, the largest rings exhibited a few very minute pigment grains, and were then usually associated with older pigmented forms.

The *later forms* of the æstivo-autumnal parasite are rather rarely seen in the peripheral circulation. Most of the Italian writers speak of their occurrence in the blood of the finger as being very unusual but not unknown. Sacharoff, in two cases of æstivo-autumnal infection, saw many rosettes in the peripheral blood. Ziemann reports that in malignant tertian cases occurring in Italy, he could follow, in the blood, the complete cycle, but that in cases occurring in Kamerun the later forms were not found in the finger blood. Plehn describes a variety of parasite which he believes is peculiar to hæmoglobinuric fever, and of which the later forms are of very small size but abundantly represented in the peripheral blood.

In five cases of the present series the entire developmental cycle of the æstivo-autumnal parasite could be followed in the peripheral

blood, and on the forms observed in these cases is based the present description of the later phases of this parasite.

After the ring has reached its full size (4 μ, 24 hrs. +), the swollen segment begins to increase in bulk and to involve a larger portion of the circumference, yielding forms seen in Plate XXX, Figs. 8-10. Some of these forms closely resembled the turban-shaped rings of the tertian parasite (Plate XXIX, Figs. 4 and 5), but were much smaller. A few fine pigment grains were usually found scattered along the periphery of the growing segment. Forms corresponding to the full-grown tertian parasite with homogeneous body were rarely seen in the peripheral blood of these five cases, but occasionally some were encountered (Plate XXX, Figs. 11 and 12) occupying three-fourths of the shrunken cell, staining homogeneously with methylene blue, and failing to exhibit a distinct nuclear body after methylene blue or hæmatoxylin. In sections of tissues from fatal cases these bodies appeared to be rather more numerous, but it was very difficult to distinguish in sections such homogeneous bodies from the slightly reticulated bodies representing the next stage of development. The fact that all other stages of the parasite were abundantly represented in the blood of these cases, while the homogeneous forms were very few, indicates that this period of development passes rapidly with the æstivo-autumnal organism.

Most of the larger forms of the parasite seen in the blood-smears of these five cases gave evidence of approaching segmentation, exhibiting a distinctly reticular structure and a condensation of pigment into one or two clumps (Plate XXX, Figs. 13 and 14). In many of these bodies the original ring persisted at one segment of the parasite, but appeared to be of reduced size and was sometimes subdivided by strands of protoplasm. The nuclear body at this period failed entirely to stain with methylene blue and was indistinct after hæmatoxylin, resembling in this respect the full-grown homogeneous tertian organism. The presence of a distinct achromatic spot adjoining the clump of pigment was very frequent in these forms and this spot was found by Nocht's stain to be composed of chromatin granules. The reticular structure of these bodies was usually distinct and the meshes were coarse.

The further development of the presegmenting forms is represented in Figs. 14 and 15, Plate XXX. In them the reticular structure becomes more distinct, the pigment is still further concentrated, and the subdivided nuclear bodies appear as small achromatic spots in the meshes of the reticulum and again stain distinctly with haematoxylin.

Rosettes (Plate XXX, Fig. 16) appeared in the peripheral blood of the five cases in moderate numbers and exhibited, in all, a very uniform structure. The pigment was grouped in a central granular clump, or, rarely, was somewhat scattered. The spores seemed to be arranged in two rows, but this appearance was probably an optical effect produced by the flattening of the more or less spheroidal body of the rosette, the spores originally lying in the central axis of the parasite falling, in the hardening process, within those lying in the periphery. When admitting of accurate enumeration their numbers were found to vary between 18 and 21. The same number of spores was repeatedly counted, in favorable specimens, smeared from the marrow of fatal cases. In sections of the tissue of fatal cases, however, the number of spores appeared to vary between wider limits, *i. e.*, 8 to 20, but as the entire rosette need not always be included in the section, the observations made in smears are the more reliable. A rim of haemoglobin invariably surrounded the rosette and strands of haemoglobin were frequently found running between the spores for a variable distance, sometimes within the outer row. These rosettes differed from tertian segmenting forms in the smaller size of the body and shrunken appearance of the cells, and in the small size, *but not in the number*, of the spores.

In none of the blood-smears nor in sections of tissues of the fatal cases were any rosettes seen without pigment. Although the arrangement of the spores and pigment often varied, there were no indications of the subdivision of the process of segmentation into the three types described by Golgi, nor were any forms seen which resembled the bodies described by Celli and Guarnieri,[1] and referred by them, with some uncertainty, to irregular sporulation.

The changes in the chromatin of the æstivo-autumnal parasite can be

[1] *Fortschritte d. Medicin*, 1889, vii, p. 528.

followed in specimens stained by Nocht's method (Plate XXX, Figs. 6-12), but on account of the smaller size of the parasite and the scarcity of older forms in the blood, it is difficult to trace the early phases of segmentation. In the young ring forms the early subdivision of the chromatin has been noted by Gautier, and in my specimens was a prominent differential character from the tertian rings. A great variety of appearances was produced by the irregular subdivision and distribution of the chromatin in the young æstivo-autumnal parasite, many of which have been sketched or described for the tertian rings. The grains were usually quite small and were sometimes apparently fused into a spindle-shaped mass, lying within the ring. Other peculiarities noted were: a markedly unequal size of the grains, a widely separate position, a frequent concentration in the centre of the ring, and, very rarely, a complete absence of chromatin.

After 24 hours' growth, the chromatin granules became more numerous and extremely minute, and were enclosed in a trace of the "milky substance," as in the tertian parasite.

When any considerable quantity of pigment gathers in the æstivo-autumnal parasite it is usually found in one or two groups, but rarely is diffuse. When the parasite has reached the full-grown homogeneous stage the pigment is commonly found concentrated in a single compact mass. This early concentration of pigment is one of the chief features which distinguish the æstivo-autumnal from the tertian parasite in the prosegmenting stage. This fact has been fully emphasized by Gautier and was very uniformly illustrated in my cases. The changes in the chromatin in the prosegmenting æstivo-autumnal body (Plate XXX, Figs. 13-15) are similar to those of the tertian parasite. In some of the specimens the chromatin granules were found in radiating lines stretching from the parent mass to the new peripheral groups (Plate XXX, Fig. 13). In many specimens the peripheral group of granules was well formed while the central portions of the body contained many diffuse granules (Plate XXX, Fig. 14). The relative quantity of chromatin in some of these bodies appeared surprisingly large. The spores in the mature rosettes usually contained single compact grains of chromatin (Plate XXX, Fig. 16), which stain readily by methylene blue, but in some rosettes

two large granules of chromatin were seen in a few spores, although the rosette seemed ready to burst. The double nuclei seen in many young æstivo-autumnal rings may perhaps be referred to the incomplete fusion of the chromatin in the rosette.

III. ON THE PLURALITY OF SPECIES IN THE ÆSTIVO-AUTUMNAL GROUP OF PARASITES.

The probability that several species of parasites are concerned in the severe types of malarial fever prevailing in warm climates, especially during the summer and autumn, has been maintained chiefly by Marchiafava, Celli, Bignami, and Grassi. From their studies of this group of fevers they divide the æstivo-autumnal group of parasites into two species: (1) The quotidian, and (2) the malignant tertian.

1. *The quotidian parasite.*—The typical fever-curve of this variety the authors found rather rarely, more frequently in relapses than in initial seizures, while a postponement of paroxysms was usually observed, and a continuous fever was very common. The typical attack is short, the fever lasting 6 to 8, rarely 12, hours, the temperature then falling to 37° C. or lower. The descriptions of the morphology of this parasite unfortunately refer only to the appearances in fresh blood.

During the rise of the temperature, the sweating stage, and the first hours of apyrexia, the blood was found to contain a variable number of red corpuscles infected with one or more very actively motile, or non-motile, parasites of discoidal or ring shape. During the afebrile period the parasite increased in size, the amœboid motion diminished or ceased, and fine pigment grains were deposited along the periphery of the organism. Later, in the larger forms, the pigment gathered in a single clump or heap of grains. During the entire development the infected red cell diminished in size and presented a "brassy" color as a result of "acute necrosis" induced by the parasite. Rosettes were seldom encountered in the blood of the finger, segmentation occurring principally in the internal organs, as seen in the aspirated splenic blood. Rarely segmentation occurred before pigmentation, but usually the numerous round or oval spores were found grouped about a central pigment mass, the rosettes being much smaller than those of the quartan or common tertian parasite. Contrary to the rule in malignant tertian infections, the young parasites were found in the blood from the beginning of the paroxysm, and, except in very mild cases, there was no

period in the cycle when the parasites were absent from the blood of the finger.

2. *The malignant tertian parasite* is distinguished by the authors on both clinical and morphological grounds. Clinically the typical paroxysm begins with a sharp elevation to about 40° C., the febrile period lasts 24 or 36 to 40 hours, is marked by a pseudo-crisis and pre-critical elevation, the fever describing, in the three-hourly chart, a characteristic course which differs from that of the mild or common tertian paroxysm. A tendency towards various irregularities is common.

In the blood the parasites may be scarce or even entirely absent at the beginning of the paroxysm. At the height of the fever the red cells contain certain small, non-motile, annular or disk-shaped bodies, or irregular amœboid bodies, which begin to show pigmentation towards the approach of the afebrile period. Most of the parasites then disappear from the peripheral blood, and rosettes are rarely seen except in some very rich infections. The prosegmenting forms are round or ovoid, are one-fourth to one-half the size of the red cell, and the pigment is gathered in a single clump or in a mass of vibrating granules. The rosettes occupy about two-thirds of the red cell and exhibit two rows of spores which usually number 10 to 12, rarely 15 to 16. The infected cells are markedly shrunken and present a "brassy" or "golden" appearance.

The authors distinguish the malignant tertian parasite from the common or mild variety on the following features:

(1) The malignant tertian parasite is smaller in all stages. (2) It assumes the ring shape, which, in the benign tertian parasite, is never seen. (This statement has been shown by many writers to be erroneous. Most of the young, mild tertian parasites are ring-shaped.) (3) Its pigment is less abundant and often non-motile, while in the other the pigment is very abundant and always in vibratory motion. (4) The rosettes are smaller, contain only 10 to 12 (rarely 16) spores (?), and are rarely seen in the finger blood. (5) The infected cell is shrunken instead of being swollen, as with the mild tertian infection.

From the quotidian parasite the malignant tertian is distinguished on the following grounds:

(1) The malignant tertian amœba is, in corresponding stages, larger and less transparent than the quotidian. (2) In the tertian parasite the amœboid movement is livelier, so that the resting discoidal forms are less frequent than with the quotidian parasite. The larger pigmented tertian forms also are often amœboid, this property persisting for 24 hours or longer. (3) The pigment of the tertian parasite is often

vibratory, but never in the quotidian. (4) In the quotidian rosettes pigment is sometimes wanting. (5) The appearance in the finger blood of a new generation of tertian parasites is seen some hours after the beginning of the paroxysm, therefore much later than with the quotidian infection.

Marchiafava and Bignami admit that the similarity between the malignant tertian and the quotidian parasites is very great, and that the differential diagnosis is very difficult, and possible only from the full-grown forms seen just before the paroxysm. They apparently do not feel quite certain that the quotidian and malignant tertian parasites are separate species, as is indicated by the following extract from their discussion on this point:[8]

"The remarkable points of resemblance between the quotidian and malignant tertian parasites make it very difficult to solve the question whether we have to do with different sorts of parasites in the strict sense, or with one and the same parasite which varies greatly in the time of its development—24 to 48 hours—and there are all intermediate degrees. On this latter theory it becomes easy to ascribe the morphological differences to the varying length of the cycle. But various facts oppose this hypothesis. First, the clinical types of the quotidian and tertian are clearly distinct from each other, and have a certain stability which is maintained in relapses and recurrences. Second, we have never met with intermediate forms or transitional cases, although it is very difficult to interpret the irregular fever. Granting that the question cannot at present be solved definitely. . . . we are inclined to adopt the view that the amœba of the quotidian and the amœba of the summer tertian are closely related varieties of one and the same parasite."

Mannaberg[9] accepts the views of Marchiafava, Celli, and Bignami, in respect to the separate nature of the malignant tertian and a quotidian group of parasites, and his description of the morphology of the parasites does not differ from that of the preceding authors whom he largely quotes. From his description of the single case of quotidian fever it is impossible to determine how many groups of parasites were present in the blood.

Grassi and Feletti[10] include all tertian parasites in one class (*Haemamœba vivax*) and state that an easily recognized species of malignant quotidian parasite (*H. præcox*) is found in Catania during the sum-

[8] Translation, *The New Sydenham Society*, London, 1894, vol. cl.

[9] Die Malariakrankheiten. Wien, 1899.

[10] *Centralbl. f. Bakter.*, 1891, x, pp. 449, 481 and 517.

mer and autumn. Their opinion appears to be based largely on an acceptance of the views of Marchiafava, Celli, and Bignami, as they offer no detailed description of these parasites nor of the cases in which they have found the latter variety.

Of the cases reported by Thayer and Hewetson, in 111 young æstivo-autumnal parasites were seen in the blood, and of these cases 73 exhibited quotidian fever. Although the examination of these cases was made almost wholly with fresh blood, it is significant that they found no evidence on which to subdivide the group of æstivo-autumnal parasites.

Ziemann, from the study of 210 cases of æstivo-autumnal infection, was unable to find sufficient grounds for the subdivision of this group. He, however, mentions the fact that the small parasite observed in cases occurring in Crema and Grosseto showed slightly less amœboid motion than that found in cases in Kamerun, and admits the possibility that quotidian æstivo-autumnal fever may be referable to a parasite which completes its development in 24 hours. He regards it as equally probable, on the other hand, that the quotidian fever is caused by the growth of two generations of small tertian parasites, but in his entire series he was unable, on account of the disappearance of the parasites from the peripheral blood, to determine accurately the length of the cycle. In his cases of malignant tertian fever, the prolongation of the febrile paroxysm, described by Marchiafava and Bignami as a characteristic feature of the malignant tertian infection, was not always to be seen. On these various grounds he concludes that all forms of the æstivo-autumnal parasite belong to one group of which the cycle varies in length from 24 to 48, or possibly 72 hours.

Gautier,[11] who has very carefully studied the malarial parasites of the Caucasus in specimens stained by Romanowsky's method, has failed to find any which he could regard as completing their cycle in 24 hours. Gautier's charts illustrating the forms of the parasite prevailing in the blood at various periods of the cycle very graphically illustrate the difficulty of following the development of the æstivo-autumnal parasite in the peripheral blood. The prolongation of the febrile paroxysm was sometimes present, sometimes wanting, and appeared to be referable to the maturation of sub-groups of parasites (see his Curve III).

In my cases there was a moderate number of pure tertian paroxysms caused by infection with a parasite morphologically identical

[11] Ueber den Parasit Laveran etc. (Russian). Moscow, 1896, and *Ztschr. f. Hyg.* 1898, xxviii, p. 439.

with the malignant tertian of Marchiafava and Bignami. The prolongation of the paroxysm and the pseudo-crisis were sometimes observed. I encountered the same difficulty experienced by Ziemann and Gautier in determining the length of the cycle from the parasites in the peripheral blood, and believe that it is seldom possible on this evidence alone to demonstrate a 48-hour cycle for the parasite. The infecting brood is seldom very compact, and in rich infections, in which alone pres egmenting bodies and rosettes appear in the blood, the groups are nearly always multiple. In my preliminary report [12] on the Montauk cases this difficulty was noted. Indeed, in 6 cases, it seemed impossible to find any marked change in the size of the parasites in the peripheral blood for three days after the chill, the ring forms persisting for that period and being constantly supplied by constantly maturing rosettes in the visceral capillaries (see also Gautier's tables). The frequency of cases showing the ring forms persisting, with slight changes, for two or three days, led to the belief that the cycle of development must sometimes extend over 48 hours. I was not then familiar with Golgi's conclusions, but since find that this observer, who is certainly qualified to know what evidence is needed to establish a 48-hour cycle, concluded that "the parasites in the blood of æstivo-autumnal cases are only an index of the infection, have little to do with the real pathogenesis of the fever, and that they represent early phases of a cycle which is much longer than has been believed."

In one of my cases, however, in which the examinations of the blood were supplemented by microscopical examination of the viscera, a 48-hour cycle appeared to be demonstrated. For the present purpose, therefore, the temperature chart furnishes by far the most convincing evidence.

The majority of my cases, however, showed quotidian excursions, and the temperature chart was of little value in determining the length of the cycle. In these the parasites in the blood, and occasionally in the viscera, were submitted to microscopic examination in the fresh condition, and in specimens stained by eosin and methylene blue, and by Nocht's method. From this study, only one feature was noted

[12] *New York Medical Journal*, 1899, lxix, pp. 144 and 149.

which could possibly serve to separate the parasites into two groups, and this related to the form of the young rings. The majority of the young æstivo-autumnal rings exhibit a single chromatin granule and a distinct thickening of one segment of the ring, and this latter character is maintained from the very smallest form up to bodies at least 4 μ in diameter. In addition to these signet-ring forms, other rings were seen which lacked the signet, were provided with two chromatin granules usually located at opposite points in the circumference of the ring, and which retained this appearance up to a considerable size (3 μ, rarely 4 μ). Such rings have been repeatedly observed before and accurately sketched (Gautier, Ziemann, Marchoux). See Plate XXX, Fig. 4.

In some severe cases these rings, lacking the signet, constituted the majority of all those seen in the blood; usually both forms were abundantly present, and in some distinctly tertian cases, while the signet-rings were more numerous, the other type was also represented in small numbers. I am, therefore, unable to conclude that these peculiar rings belong to a separate species of parasite with a short cycle of development.

No other morphological differences were noted in the young rings of these cases. It is possible, but hardly probable, that the quotidian parasite, if it exists, was not represented among the cases seen at Montauk.

Between parasites of larger size, as seen in the blood and in smears of the viscera of fatal cases, considerable difference in size was noted, but the smallest presegmenting bodies and rosettes encountered were found associated only with the usual form of signet rings. My observations on the parasites in the fresh condition failed to show any uniform difference in the refractive quality or amœboid activity, but under the circumstances they could not be pursued so extensively as was desired.

The evidence secured failed therefore to establish any clinical or morphological grounds on which to separate the parasites of pernicious malarial fever into two or more groups.

It does not seem likely that such a division can be successfully maintained except on morphological grounds. The difficulty in fol-

lowing the development in the blood of even a 48-hour parasite is well illustrated by Gautier's tables, and must be considerably increased when dealing with a more rapidly maturing form. The tendency of the æstivo-autumnal parasite to be held in the viscera during its later phases very greatly complicates the undertaking. In the cases of quotidian infection reported in detail by Marchiafava, Bignami, and Guarnieri, one fails to find, in the results of the blood examination, convincing evidence of the existence of one group of parasites with a 24-hour cycle. The clinical peculiarities observed in these cases are not without significance, but are of themselves entirely inconclusive and still require confirmation. The evidence on which the quartan, tertian, and æstivo-autumnal parasites are separated is of entirely different value from that on which it is proposed to divide the æstivo-autumnal group. Distinct morphological characters have not been clearly established, peculiar clinical features have not been shown to characterize any considerable group of cases, the developmental phases of a 24-hour cycle have not been demonstrated in the blood or viscera, and it would seem that further observations are required before the existence of a quotidian parasite can be accepted even as a working hypothesis.

The later position of Marchiafava and Bignami, admitting that the existence of two species in the æstivo-autumnal group is not proven, would therefore seem to be justified.

That 72 hours may occasionally be required for the cycle of the æstivo-autumnal parasite is indicated by the observation of a few cases of quartan fever with this infection. Such cases have been reported by Gautier and Ziemann. The paroxysms, in Ziemann's case, were twice repeated, with intervals of two days; the fever almost completely subsided in the interim; and there seems to be no reason to suppose that the irregular maturation of tertian broods could possibly have produced the paroxysms.[13]

[13] R. Koch, whose report on malaria in tropical countries has appeared since the completion of this article, has also reached the conclusion that there is but one species of the æstivo-autumnal parasite, and that in fresh cases the fever is uniformly of the tertian type, but later tends to become more and more irregular. He considers "tropical" a more appropriate epithet than "æstivo-autumnal" to designate this parasite and the fever caused by it (*Deutsche med. Wochenschr.*, 1900, p. 781).

Haemamoeba immaculata.

Grassi and Feletti, Marchiafava and Celli, Bignami, Guarnieri, Sacharoff, Marchoux, and Ziemann, report cases in which rosettes free from pigment were found in the blood or viscera. Most of these authors, while admitting that the parasite may occasionally sporulate without producing pigment, are not inclined to regard *Haemamoeba immaculata* (Grassi) as a separate species.

Grassi and Feletti, however, claim to have observed in a bird pure infection with a variety of parasite which failed to produce pigment, and regard the appearance in the human subject of rosettes without pigment as evidence of infection by a distinct species of parasite. Mannaberg also accepts this view.

In the report of the examination of the viscera of this bird by Grassi and Feletti no mention is made of the presence or absence of pigment, and it is impossible to determine whether or not the infection had failed to produce pigment in the viscera as well as in the peripheral blood. In all cases in which pigment-free rosettes have been found in the blood of human subjects there have been found the usual pigment deposits and pigmented rosettes in the viscera. That there is considerable variation in the quantity of pigment produced by the parasite in fatal cases is shown by the reports by Marchiafava and Bignami of fatal cases in which a microscopic examination was required to show the presence of very scanty deposits in the viscera. Ziemann mentions in this connection that he has seen a presegmenting body of the benign tertian type which was entirely free from pigment.

I have already mentioned (p. 447) the marked resemblance which red cells, harboring five or six parasites, may bear to pigment-free rosettes. Most of these rosettes, as described, contained a small number of spores (6 to 10). In the sketches of Marchiafava and Celli, as noted by the authors, the spores of the pigment-free rosettes are of unusually large size. Their appearance is almost identical with the cell harboring seven young parasites sketched in Plate XXX, Fig. 2, from the marrow smears of a fatal case. If the latter cell had been found in a section of tissue it would have been scarcely possible to distinguish it from a rosette without pigment.

In a drawing accompanying the article of Bastianelli and Bignami,[14] is a nearly normal red cell apparently infected with six young parasites, in explanation of which the authors suggest an irregular form of segmentation. If the drawing is accurate, it appears to me that the cell

[14] *Boll. d. r. Accad. med. di Roma*, 1894, xx, p. 151, plate i, fig. 26.

is too little altered to have long harbored a growing parasite, and that the young parasites are too large for spores. The drawings of the cerebral capillaries, filled with rosettes without pigment, hardly admit, however, of this interpretation. Nevertheless, a failure of the human malarial parasite to produce pigment is such a violent departure from its ordinary physiology that the fact should rest only on the most absolute proof, and it may not be amiss to have suggested a possible source of error in this field.

In any case the grounds are insufficient to warrant the classification of *Hæmamœba immaculata* as a separate species of parasite, and seem at best merely to justify the opinion of other observers that pigment-free rosettes, as seen in the human subject, are an occasional form of the æstivo-autumnal parasite. This opinion is well set forth in the words of Marchiafava and Bignami:[15] "We cannot allow that a distinction should be drawn between the *Hæmamœba præcox* (tertian parasite of Grassi) and the *Hæmamœba immaculata*, as two separate species. Segmentation with no pigmentation has been observed by Marchiafava and Celli, but only in very rare cases, and always together with pigmented rosettes. So that in these cases it would be necessary to suppose a double infection, an hypothesis that is devoid of all foundation. We shall feel unable to change our opinion until we meet with cases which show no trace of melanæmia and which, therefore, mean a pure infection with the *Hæmamœba immaculata*."

IV. THE NUCLEAR BODY OF THE MALARIAL PARASITE.

From the earliest period of the minute study of the malarial parasite certain structures in its body have been recognized as probable nuclear elements, but the exact significance of these structures and their relation to the definite nuclear elements of metazoan cells have never been fully determined.

In 1889, Celli and Guarnieri,[16] from the examination of fresh malarial blood stained by methylene blue in ascitic fluid, described in the larger parasites, an outer deeply staining ectoplasm and an inner nearly achromatic endoplasm. In the lightly colored endoplasm, surrounded by a narrow, perfectly achromatic zone, was a sharply marked body of variable structure, sometimes compact, sometimes reticulated, but evi-

[15] New Sydenham Society's Translation, op. cit.

[16] References to the authors here cited will be found in the monographs of Thayer and Hewetson, of Ziemann, and of Mannaberg, already quoted.

dently representing the nucleus of the parasite. On the inner border of the ectoplasm of younger parasites they found a deeply staining body which they regarded as the early form of the nucleus. Their plates accurately depict the growth of the "endoplasm and nucleus" up to the presegmenting stage, into which they were unable to follow it. This demonstration of the nuclear body was, in the minds of competent observers, the beginning of the end in the controversy regarding the truly parasitic nature of the malarial organism.

Using the same technical methods, Grassi and Feletti, in 1890, described in the larger parasites "a large vesicular nucleus such as is seen in many rhizopods." This nucleus was usually eccentric, possessed a very thin, often indistinct membrane, and an intranuclear network filled with a semi-fluid substance. The intranuclear network exhibited a nodal thickening resembling a nucleolus, which was sometimes round or often showed several radiating filaments stretching toward the nuclear membrane. This nucleus was not found in young parasites. The authors believed they could discover evidences of direct division of the nucleus, beginning 12 to 16 hours before segmentation of the body. The nucleus of Grassi and Feletti undoubtedly corresponds to the endoplasm of Celli and Guarnieri.

In 1891 Romanowsky published his observations on the structure of the tertian parasite as demonstrated by his special staining method. He described the nucleus as a colorless central area in the parasite, in which appeared a smaller body staining of a carmine violet color, the "nucleolus" or chromatin of the nucleus. In the larger parasites he described the development of fibrillar chromatin bodies in the nucleus, indicating a process of indirect division. These filaments were indistinct but gave the mass of chromatin a less compact appearance. The diaster stage is roughly indicated in one of the sketches. Romanowsky described, also, "quinine forms" in which the clear zone of the nucleus was wanting, this structure fading insensibly into the body of the parasite, while the chromatin was subdivided into many fine granules. To judge from the drawings, these "quinine forms" appear to be identical with Gautier's presegmenting forms (see p. 440). Romanowsky mentions no stage of the parasite which failed to show a nuclear body stainable by his method.

Sacharoff, in 1891, observed the "nucleolus" lying in a clear nucleus in specimens of æstivo-autumnal parasites stained by gentian violet, and noted the disappearance of the nucleus just before segmentation. In 1893 he applied Romanowsky's method to the minute study of the nucleus and described a fibrillar structure which occasionally showed karyo-

kinetic figures. He found, further, that the flagella of the parasite stain like the chromatin of the nucleus, and concluded that the flagella are separate chromosomes of the karyokinetic nucleus, extruded from the parasite under the influence of cold. In 1895, he reported a further study of the nucleus, and described the "extrusion of chromosomes" (exflagellation) from the malarial parasites of young crows. The parasites of these animals were found specially adapted to the purpose, as their nuclei are large, chromatin filaments are distinct, and flagellate bodies are found in the blood immediately after shedding. In these parasites he depicts intracellular formation of flagella and the extrusion of all the chromatin from the body of the parasite in the form of flagella. The author refers (1895) to the studies of Sala on the eggs of *Ascaris megalocephala*, in which indications were found of an active movement on the part of the chromosomes, and to the conclusion of Strasburger that the changing position of chromosomes in some vegetable cells results from an active movement on the part of these structures. It may be added that in recent years an extrusion of all chromatin in the form of flagella has been observed in various forms of coccidia by several investigators.

Bastianelli and Bignami described the minute structure of the æstivo-autumnal parasite in specimens stained by hæmatoxylin. In the young parasite they describe as "endoplasm" the large central achromatic area through which shines the hæmoglobin of the infected cell, while the deeply staining peripheral granule was said to consist of chromatin. This nuclear body, or endoplasm, possesses no membrane and exhibits no special structure. The chromatic granule increases in size as the parasite develops and the clear endoplasm acquires a light bluish tinge, partly obscuring the hæmoglobin. Later, when the pigment has gathered in a single mass, the body of the parasite becomes homogeneous and the chromatic granule disappears. These changes mark the beginning of the reproductive phase, and may be followed very shortly by segmentation. During segmentation the chromatin reappears scattered through the body in fine particles, about each of which a ring of chromatophilic substance gathers. A small remnant of the endoplasm is left unutilized in the segmenting process. The spores at first contain no endoplasm, which appears only in the young parasite. The authors do not find, either in their own preparations or in the drawings of others, any definite structures recalling a true nucleus. The disappearance of the chromatin before sporulation they find to be analogous to a similar phenomenon in the Gregarinidæ and Coccidia, while in the Oscillariæ one or more disseminated granules of chromatin represent

nuclear bodies similar to those seen in the malarial parasite. The nucleus of the malarial amœba, they believe, never assumes the vesicular or resting stage on account of the rapid succession of generations. In the crescents, with rare exceptions, they found no chromatin, and therefore regarded these bodies as sterile.

Mannaberg (1893, 1899) followed the development of the nucleus, as described by Celli and Guarnieri, in specimens stained by hæmatoxylin, and by a special procedure of his own. He was unable to find evidence of a karyokinetic division of the nucleus.

Ziemann studied the structure of the tertian, quartan, and æstivo-autumnal parasites by means of his modification of Romanowsky's stain. He followed minutely the changes in the nucleus in each variety, and described many minor variations which may be of value in differential diagnosis. At a rather early period of the cycle the solid chromatin granule was usually found to become less compact and was sometimes divided into two or three portions. With the disappearance of amœboid motion in the parasite the chromatin is usually divided into many fine filaments or spindles from which are derived, during the full-grown and presegmenting stages, an increasing number of secondary chromatin bodies of variable position and contour, but eventually forming the nuclei of the young spores. He found the eccentric position of the nucleus in the young tertian and its central position in the quartan parasite to be very constant differential characters in these organisms. In the "full-grown" stage of the parasite he found the chromatin more difficult to stain. The parasites which failed to exhibit a mass of chromatin he regarded as sterile. He first described appearances which recalled the chromatin filaments and mitotic figures of Romanowsky, but later concluded that no distinct traces of a true karyokinetic process could be demonstrated in these parasites. After a growth of 16 to 24 hours, the chromatin mass was found to break up into a number of spindle-shaped granules, which showed a very inconstant arrangement. Meantime the limits of the nucleus became very indistinct. In many parasites the nucleus and chromatin disappeared, the parasite increased markedly in size and presented a rich deposit of pigment grains in active vibratory motion. These forms he regarded as sterile, and included among them the elliptical and large oval bodies, in most of which he was unable to demonstrate any traces of chromatin. In a few crescents obtained from the bone-marrow, 11 hours after death, he was able to demonstrate a more or less compact mass of chromatin, but always of reduced bulk.

Okintschitz has described the nucleus of the young forms and the fine

structure of the malarial parasite in specimens stained by eosin, methylene blue, and safranine. In the young parasite the nucleus was found to be a compact mass which, in the tertian parasite, failed to stain with methylene blue, but in the æstivo-autumnal variety stained densely with this dye. The further changes in the nucleus were not fully traced.

Marchoux describes the nucleus of the æstivo-autumnal parasite of Senegal in specimens stained by eosin and methylene blue and by a mixture of thionin and carbolic acid (see p. 431). In the early ring-shaped organism the enclosed substance was regarded by the author as the nucleus, the deeply staining eccentric body as the nucleolus. Sometimes two nucleoli were found at opposite poles of the parasite, an appearance which he was inclined to refer to a process of conjugation in view of the fact that later phases exhibited a single nucleolus. In the full-grown stage the nucleolus assumed a position in the centre of the nucleus, gradually dividing into a number of smaller bodies arranged in the form of a wreath. The later changes were not followed, as the parasites disappeared from the peripheral blood.

Gautier, in 1895, reported a study of the malarial parasite of the Caucasus in specimens stained by Romanowsky's method. He finds that the nucleus consists of a vesicular portion and a violet-staining mass of chromatin. The chromatin body is usually surrounded by a narrow, "milky zone," which is sometimes continued about the entire vesicular nucleus. In the ring stage the haemoglobin shines through the vesicular portion of the nucleus. In many parasites at various stages the "milky zone" is invisible. With the beginning enlargement of the body of the parasite the chromatin changes from a small compact body to a less compact oval mass of granules. It sometimes early breaks up into two or three portions, or it may consist of a single mass of small granules. In some of Gautier's drawings these granules are placed in the centre of the haemoglobin mass which he regards as shining through the vesicular nucleus. In bodies probably representing the early presegmenting stage of the parasite he describes the development of a reticular structure of the parasite and the total disappearance of chromatin particles. Later the chromatin grains reappear in the meshes of the reticulum. In crescents and ovoids he found numerous small chromatin granules. In the crescents these granules appeared to be much more minute than in the ovoids. Many large parasites without chromatin he regarded as dead.

The nuclear changes which I have observed in specimens stained by Nocht's method have been detailed under the descriptions of

species. They were largely in accord with the observations of Gautier and of Ziemann.

I find that the nucleus of the parasite belongs to the "distributed type" of protozoan nuclei (p. 436), consisting of granules of chromatin and, certainly in the older and possibly in all stages, of an achromatic substance in which the granules are embedded. While the claim of Bastianelli and Bignami must be admitted, that the parasite possesses "no true nucleus," in the metazoan sense, it exhibits nevertheless all the nuclear structures required in some protozoa.

Neither the nucleus nor the achromatic substance appears to be necessarily connected with the interior of the ring, which is the form assumed by the young and vegetative parasite. It seems most probable that this form represents a true ring, or if not, the ring is bridged by a substance which has no essential nuclear relations.

I find no forms in the fertile cycle of the parasite in which chromatin cannot be demonstrated. Various intra-cellular and extracellular forms devoid of chromatin are for that reason necessarily regarded as sterile.

Although there appears to be abundant analogy in the nuclear changes in the parasites of birds and in some closely related coccidia, to indicate that the human parasite may divide by a modified form of karyokinesis, I could find no sufficient ground for applying this term to the series of nuclear changes observed in the presegmenting parasites in man.

Labbé, Danilewsky, Sacharoff and others, in the blood parasites of animals, and Simond and Siedlecki, in various coccidia, find that the chromatin regularly appears at some stages in the form of fibrils, and that these may describe figures rather closely resembling the mitoses of metazoan cells. Romanowsky claimed to have seen distinct chromatin filaments, and sketches imperfect mitotic figures in the tertian parasite. Ziemann relinquishes a similar claim in his second article, admitting that no distinct mitotic figures are to be demonstrated in the human parasite. Gautier's sketches show nothing of these filaments. In my specimens from fresh malarial blood chromatin was never seen in the form of a filament, all elongated masses being invariably of granular structure. On the other hand, when exflagel-

lation occurs with the human parasite, the chromatin becomes filamentous, figures resembling monasters are produced, and the chromosomes are extruded as active flagella. This process is entirely in accord with the changes depicted by Sacharoff in the parasites of birds.

It appears, therefore, that in the fertile cycle of the malarial parasite division occurs by a very simple process which may be likened to amitosis, the only visible changes in the chromatin being subdivision and fusion. In another cycle of development, adapted for the extracorporeal growth of the parasite, division occurs by a modified form of karyokinesis, the chromosomes leaving the parent cell to fertilize other individuals. Some of the structures seen in coccidia by Simond and Siedlecki have been interpreted as showing the fertilization of one parasite by the flagellum of another. MacCallum,[17] working with the blood of infected crows, repeatedly saw free flagella enter other parasites in which they became lost, and he was able to repeat this observation in a case of human æstivo-autumnal infection. Evidence is therefore gradually being gathered to determine the true significance of flagellation and to locate in the proper place the function of karyokinesis in the malarial parasite.

V. THE CRESCENTIC BODIES.

While the results of recent studies of the coccidia (Simond, Siedlecki, Schaudinn) bear on some obscure points in the biology of the malarial parasite, the full significance of the crescentic bodies, even in the coccidia, has not yet been demonstrated, although the position of these bodies in the developmental cycle has been determined. In various coccidia it has been shown that there are two cycles of development, one, the sporulating cycle, leading to the development of encysted bodies, the other, asporulate and parthenogenetic, leading to the development of crescentic and flagellate bodies. The individuals of the sporulating series are capable of reproduction in the host, but in the asporulate series, the crescents and flagellate forms are very fragile, disappearing rapidly when exposed to unfavorable conditions, and alone are incapable of self-perpetuation. There is evidence in the coccidia that some of the crescentic bodies represent the female element and require fecundation by the flagellum or male element, in order to become fertile.

[17] Journal of Experimental Medicine, 1898, iii, p. 117.

MacCallum's observations on Halteridium, a crescentic parasite of birds, indicate that these crescentic bodies are of two varieties, one, the male, producing flagella, the other, the female, uniting with a free flagellum and developing into a motile form called the "vermiculus."

Further evidence on this point has been furnished by Ross, who found that when the blood of birds infected with Proteosoma, a species of parasite closely resembling the malarial amœba, reaches the stomach of the mosquito, many of the organisms become flagellated. A few days later he finds in the stomach-wall of the mosquito certain large encysted pigmented bodies containing many rod-like structures and some "black spores," which on the rupture of the cyst gain the general circulation. In the salivary glands of the insect these "germinal rods" may be found in large numbers. Ross was able to infect young birds by subjecting them to the bites of mosquitoes fed on blood containing Proteosoma, but was unsuccessful with Halteridium.

Grassi, Bignami, and Bastianelli, have confirmed and extended Ross's observations. These investigators succeeded in conveying the æstivo-autumnal infection from one human being to another by means of a particular variety of mosquito, Anopheles claviger, the "dapple-winged" mosquito described by Ross. Moreover, they fed their mosquitoes on blood containing crescents, showing that these bodies are capable of further development in a new host. In the mosquito they observed, as Ross had done, exflagellation of the crescents, development of encysted bodies in the stomach-wall, discharge of "germinal rods," and their accumulation in the salivary glands of the infecting insect. Later they succeeded in transferring the tertian parasite in the same way, the large hyaline forms furnishing the flagella in the mosquito's stomach. They found no bodies resembling the vermiculus of birds, and it has not been shown how the parasite pierces the wall of the stomach. It is thus clear that the crescentic body is a form of the parasite adapted to further development in a new host.

Of the mode of origin of the crescents in man there is still nothing definitely known. In support of Mannaberg's theory that they are conjugation forms resulting from the union of two ring forms, no new facts have been observed. Grassi and Feletti, who, in 1891, claimed that in birds a certain number of crescents were undoubtedly produced by conjugation of younger forms, have apparently not insisted upon the correctness of this view. On the contrary, the studies of the development of coccidia in the rabbit, salamander, cuttle-fish, and other animals, strongly oppose Mannaberg's theory, for in these organisms, which, according to Metchnikoff, are clearly related to the malarial parasite, the

crescentic form is produced in an entirely different manner, by the segmentation of a large spheroidal body into several small but fully formed crescentic bodies. No similar parent bodies have been described in the blood or tissues of the human subject.

Celli and Guarnieri, in 1889, and Canalis in 1890, depicted the young forms of the crescentic bodies as small, narrow crescents with considerable fine pigment, lying within slightly altered red cells, and traced the development through a gradual increase in size, with destruction of the haemoglobin of the infected cell, up to the adult crescent. From the adult crescents, ovoid, elliptical, and spheroidal bodies may then form, and these frequently become flagellated. These phases of development have been very generally accepted, and are largely in accordance with analogous processes in various coccidia. There is, however, a lack of agreement regarding the relation of the ovoid and spheroidal bodies to the crescentic forms. When examined in the fresh condition crescents are frequently seen to assume the spheroidal form, and if a little moisture is added the spheres may extrude flagella. Occasionally, however, the spheres or ovoids may be seen to revert to the crescentic form, as described by Ziemann and others. Now, crescents of almost any size may be made to assume the spheroidal form, from which it appears that this body is not always to be included in the natural developmental series of the crescent. I have seen spheroidal bodies develop from crescents about which there was hardly a trace of haemoglobin, while in other cases the spheroidal body did not occupy more than two-thirds of the red cell. The young crescents which appear in the blood on the fourth or fifth day of the paroxysm have, in my cases, been of small size, rather broad, and often no longer than the red cell. They are often distinctly oval or spheroidal in the stained specimen. During the fifth to the seventh days they gradually increase in size, with progressive destruction of haemoglobin, and finally assume the elongated crescentic form, without haemoglobin. My conclusion, therefore, is that the ovoid and spheroidal bodies seen in the ordinary stained specimen are usually younger forms than the elongated crescent, and that the spheroidal bodies which form in shed blood may be derived from crescents of almost any age. The quantity of haemoglobin about the spheroidal

body would seem to be a reliable indication of the age and original form.

In 1889 Canalis described a form of *segmentation* in crescents. The segmenting bodies were elliptical in form and discharged eight or ten rather large spores. At the same time, Celli, and Marchiafava and Golgi, were inclined to believe that crescents might sporulate in the blood, but were not certain that they had ever seen such forms. Antolisei and Angelini, in 1890, confirmed Canalis's observations, stating, however, that the new spores possess a double contour. Grassi and Feletti, and Sacharoff, believing that the crescents represent a separate species of organism (*Laverania malaria*), accept of necessity the hypothesis of their sporulation, but have not positively identified segmenting forms in the blood. Lewkowicz (1897) reports that he has seen two crescents in the act of sporulation in the blood, and in the splenic blood he describes some segmenting crescents containing as many as thirty spores. The transverse subdivision of crescents has been observed by Grassi and Feletti, Mannaberg, Ziemann, and others. Ziemann, however, regards this process as unquestionably not of a reproductive nature. The transverse segmentation of crescentic bodies has been clearly demonstrated in coccidia (Jackson Clarke), but the fate of the segments is not shown, and while there are indications that the crescentic bodies of coccidia may be multiplied to some extent in this way, the same evidence clearly shows that the process is exceptional.

The various phases of gemmation and budding described by many writers have never been strongly urged as a natural method of reproduction of crescents.

Accordingly, opposed to a moderate number of inconclusive and often uncertain observations favoring the segmentation of crescents, there are entirely negative results from the vast majority of observers.

Various studies of related protozoa indicate that crescentic bodies, after fertilization, regularly proceed to further development with encystment and the production of an entirely different form of the parasite, but sometimes leading to autoinfection of the same host. Although there is no satisfactory evidence that the malarial crescents can develop further in the human being, it is by no means certain that their formation and development are entirely innocuous to the patient.

Not a few observers have connected certain febrile paroxysms with the growth of crescents. Golgi in 1889 referred some forms of fever at long intervals to the development of new broods of crescents. Laveran still (1899) holds that crescents alone, without the presence of other forms, can be associated with a febrile paroxysm. Canalis, who claimed to have found sporulating crescents, connected the segmentation of these bodies with paroxysms recurring in three or four days. Celli and Sanfelice, and Grassi and Feletti not infrequently observed paroxysms in birds associated with the appearance of crescents only in the blood. Lewkowicz believes that the development of crescents may produce quotidian or tertian fever, or paroxysms at long intervals. He believes, also, that crescents are not so refractory to quinine as is generally supposed; that they disappear under quinine after a variable period, and that the long-persisting forms are new individuals reproduced in the viscera from day to day.

Most authorities, however, prefer to attribute the irregular paroxysms to the production of a limited number of ordinary amœboid parasites, which sometimes fail to reach the general circulation in demonstrable numbers.

The possibility that the development of crescents is associated with a febrile paroxysm is by no means disposed of by the proof that crescents do not sporulate. There is almost certainly a secondary cycle of development of the parasite leading to the formation of crescents, and this cycle may well be several times repeated, each time with fever. For each crescent destroys a red cell, and the crescents in the blood are sometimes as abundant as the brood of young amœbæ. Although most cases of fever at long intervals are probably simple relapses, it is impossible to deprecate wholly the tendency to regard some of these paroxysms as evidence of a second cycle of development in the parasite leading to the formation of crescents. In the Montauk series I was frequently surprised to find only young crescents in the blood associated with mild seizures at irregular intervals. It was especially noted in some cases in which crescents persisted in the blood after fever had subsided and while quinine was still being administered, that shortly after a mild chill numerous young crescents appeared in the blood.

Some *morphological features* of the crescentic bodies are still of

active interest. The early observers, who believed that crescents were an encysted form of the parasite, frequently depicted these bodies with a distinct double contour, representing a sharply defined membrane. Without entering into the details of opposing views, it may be said that this extreme claim of a distinct double membrane has been slowly abandoned, and the most that is maintained is the existence of a condensed outer border about the crescent. I find that this outer border may be colored red by strong staining with eosin. That the reddish border thus developed is not identical with the membrane of the red cell appears from the fact that it invests the concave side of the crescent where it may be widely separated from the projecting bow of the red cell. The remnant of the red cell which stretches like a bow across the concavity of the crescent, while usually single, appeared double in one of the specimens. The ends of the bows then overlapped, each enclosing a little more than one-half of the crescent (Plate XXX, Fig. 23).

The identity of the bow with the membrane of the red cell has been accepted without question, and its development in young specimens leaves little doubt of this origin, but why it should increase in dimensions with the growth of the crescent and why it is occasionally double are at present obscure questions.

The application of Nocht's method to the crescentic bodies furnished valuable additions to the knowledge of these forms. Ziemann found the vast majority of crescents to be entirely free from chromatin. Gautier, however, working with Romanowsky's procedure, was apparently the first to demonstrate the presence of chromatin in any large proportion of crescents in human blood. In the young ovoid or elliptical forms he found a well-marked group of rather large granules of chromatin. In the full-grown crescent a single group of very fine granules lying in the centre of the body and often partly obscured by the pigment mass, could, in the majority of specimens, be fully identified.

With Nocht's method I have been able to demonstrate chromatin granules in the vast majority of crescents in all stages (Plate XXX, Figs. 17-23). In the younger forms the granules were usually larger and more distinct than in the older forms. In the adult crescents the chromatin was usually found in a single rather compact mass of

minute granules, which was usually much obscured by overlying pigment. Occasionally the chromatin mass lay to one side of the wreath of pigment, in which case it was very easily identified. In a few specimens in which the pigment was diffusely scattered over the crescent, the chromatin was very clearly visible (Plate XXX, Fig. 22).

Two groups of chromatin granules were seen in a moderate number of crescents, in some of which the pigment was arranged in the form of the figure 8. In these specimens both groups of chromatin granules were inclosed in a single mass of clear achromatic substance (Plate XXX, Figs. 21 and 23).

In a few specimens it was impossible to detect any traces of chromatin, indicating that these particular forms were sterile. Such forms, however, were not more numerous than were chromatin-free parasites in ordinary cases of tertian or æstivo-autumnal infection, while they were difficult to find in specimens in which the staining had been especially successful.

Nocht's stain very clearly demonstrates an elliptical relatively achromatic area in the centre of the crescent in which the pigment and chromatin are usually included. The line of demarcation between the bluish staining poles and the achromatic area was often very sharp after the application of this method. Occasionally the achromatic area was found well cut in one pole (Plate XXX, Fig. 20).

The application of Nocht's method to other forms of the parasite greatly increases the number of forms in which evidences of approaching segmentation may be found. In my specimens of crescents no variation in the chromatin granules or mass was detected pointing to a reproductive process. The older and larger the crescent the smaller and less distinct these granules became. In some spheroidal bodies of a fatal case, however, the body of the parasite was distinctly reticulated, although the chromatin grains remained in a single mass.

VI. EXTRA-CELLULAR PARASITES.

That the young parasite during its passage from the parent rosette to the new red cell is sometimes caught in the plasma in both fresh and dry specimens is evident from the reports of various observers.

The possibility of identifying such young free forms in the fresh

condition may, however, be doubted. Ziemann, commenting on this point, says that "only in the beginning of his studies of malaria did he venture to identify young amœboid organisms in the plasma of fresh blood." Celli and Guarnieri (1889) have sketched the appearance of young extra-cellular bodies, including forms of at least two species of parasites, in fresh blood stained by methylene blue after their special procedure, but many of these, especially the pigmented ones, were undoubtedly separated from the cell during the manipulation. In preparations of fresh blood, parasites so frequently pass from the cell into the plasma that it may be doubted if any accurate estimate of the number of extra-cellular forms in the circulating blood can be obtained by this method of examination. In some cases in which I have seen suspicious extra-cellular bodies in fresh specimens, Nocht's stain failed to show any extra-cellular parasites whatever. If reliance be placed upon dry specimens stained by this method, and the demonstration of a distinct nucleus be required, extra-cellular parasites must be looked upon as a comparative rarity, but may undoubtedly be seen in exceptional cases. Gautier and Ziemann depict such forms, while mentioning their rarity. Romanowsky could find young extra-cellular tertian parasites only in patients taking quinine. The same rule appears to hold with the later stages of the parasite, extra-cellular parasites being found with extreme rarity. In fresh specimens a considerable number of large forms appear to be extra-cellular, but these, in stained specimens, usually show some enclosing remnant of a red cell.

Various *sterile forms* of parasites described at length by Golgi, Ziemann, Bignami and Bastianelli, and others, while usually endoglobular, are sometimes seen in the plasma, and in dry specimens may be found to be distinctly extra-cellular. The characters of these sterile forms are, according to Ziemann: (1) increase in size, (2) loss of amœboid motion, (3) greater abundance of pigment and increased vibratory movement of pigment granules, (4) markedly hyaline appearance in stained specimens, (5) complete or nearly complete absence of chromatin. Ziemann, however, includes among the sterile forms crescents and spheres derived from crescents, which do not properly belong in this class, as they contain chromatin and under

suitable conditions are capable of further development. Other sterile forms described by Ziemann were found most abundantly in the splenic pulp after death. These were spheroidal bodies, of large size, hyaline aspect, vibratory pigment, and deficient supply of chromatin.

Similar large forms have often been described as derived from full-grown quartan and tertian parasites, and their extrusion from the red cell has been followed in specimens of fresh blood. The extra-cellular position of most of these forms appears therefore to be artificial. Working principally with dry specimens, I have always had great difficulty in finding any of these large extra-cellular forms, and believe that they are extremely rare in the circulating blood. In specimens of fresh blood, however, which have been allowed to stand for 1 to 24 hours such forms become rather numerous.

Vacuolation has also been frequently described in these large sterile parasites. In the earlier observations of parasites in the fresh condition the nuclei were sometimes mistaken for vacuoles, an error against which Golgi warns. In stained specimens I have very rarely been able to identify vacuolated parasites and believe that their identification in fresh blood is usually very hazardous.

The relation of the parasite to the red cell still remains a matter of dispute. Laveran holds that the majority of parasites are merely attached to the surface of the cell, though some are found within its substance. The crescentic bodies he regards as strictly intra-cellular.

The frequent appearance of the projection of the æstivo-autumnal ring beyond the circumference of the red cell has led many to believe that this parasite, at least in its early stages, is merely attached to the cell. Marchiafava and Bignami, however, point out that the æstivo-autumnal ring in the fresh condition never sends pseudopodia beyond the edge of the cell, and may be seen dipping down or swimming at different levels in the cell.

Gautier very accurately depicts the appearance of the ring projecting beyond the cell, and there seems to be no good reason to doubt that such parasites are merely attached to the cell. It by no means follows, however, that later stages of the æstivo-autumnal ring are not found within the cell, as described by Marchiafava and Bignami. It is generally accepted that the tertian parasite lies within the red cell,

yet in many tertian cases the body and especially the nucleus of the parasite appear to project beyond the border of the cell, even more distinctly than in the case of the æstivo-autumnal ring. Such attached forms seem to be more frequent in the actively amœboid stage, and in cases taking quinine. In fresh specimens which have been allowed to stand and are afterwards dried and stained, the parasite may be found in various stages of extrusion, and similar appearances of projection of body and especially of nucleus beyond the cell are numerous. Mannaberg probably correctly expresses the facts in this matter as follows: "The young parasites swim in the plasma for a very short time and soon become attached to red cells. They remain attached to the cell for a time but soon penetrate within, where their further development is completed."

It is probable that the æstivo-autumnal parasite remains attached to the cell longer than the tertian, possibly because it is less actively amœboid..

VII. ON A FORM OF CONJUGATION OF THE TERTIAN MALARIAL PARASITE.

In four cases of tertian infection I have encountered appearances in the blood which seem to admit of no other explanation than that of conjugation of malarial parasites. In a considerable number of other cases similar appearances were found, but much less frequently.

The blood in these cases showed a moderate number of young rings and a large number of half-grown and full-grown forms. A great many red cells showed double infection with young rings. In many instances these rings were entirely separate, each exhibiting a single large granule of chromatin. Many cells, however, contained two rings, which were clearly *fused together along one segment of the ring*, and two large chromatin granules were then invariably found at different points in the rings (Plate XXXII, Figs. 2-5). The fused parasites usually differed in appearance. One was a large delicate ring with a thin bow, and chromatin granule of moderate size, while the other was a coarser body with thickened bow, enclosing little or no hæmoglobin, and exhibiting a large chromatin granule (Plate XXXII, Figs. 3-5). These differences between the two conjugating parasites could not always be found. Among the single rings, the

two forms of young parasites were often distinguished, but no single rings could be found containing two *equally* large chromatin granules, while every red cell that exhibited two large and equal chromatin granules contained also two distinct rings. It appeared therefore that the bodies of many parasites had become fused together, while their nuclei remained separate. Occasionally the two chromatin granules were found close together, but no distinct signs of a fusion of chromatin were found at this stage.

On examining the parasites in later stages of development, most of them were found to have lost the ring form, and to have spread out into a large number of threads, with nodal thickenings, variously curled in the red cell. These threads evidently represented the pseudopodia of a very active amœboid stage. The chromatin masses were now subdivided into 10 to 12 granules, but in the majority of the cases these masses were far apart and showed no tendency to unite. In many cells, however, the amœboid figures were less marked, *and the masses of chromatin lay side by side united by a little achromatic substance*. Later some parasites were found in which the two groups of rather large chromatin granules lay in *immediate apposition, surrounded by achromatic substance*. This phase was marked by a distinct reduction in the length of amœboid figures (Plate XXXII, Figs. 6-11).

Many older, spheroidal, hyaline forms, belonging to this same brood, were found in these cases. All the older hyaline forms were single and exhibited a single large group of fine chromatin granules. Not one cell harboring two full-grown parasites could be found in prolonged and repeated search through several slides.

The question therefore arises, what became of the very large number of twin parasites seen in all the younger stages? In one of the Montauk cases the two broods were of different ages, one approaching segmentation *and all single*, the other less than half-grown and *almost invariably twinned*. Can twinning occur in part of a brood and not in its oldest members, or in one brood extensively and not at all in its predecessor? While such physiological variations are possible, they appear extremely improbable, and one is forced to the conclusion, merely from the absence of older twinned parasites, that conjugation

occurred. Whatever interpretation may be placed upon this peculiar absence of older twinned forms, the finding of all stages of union, first of the bodies, later of the nuclei, as illustrated in Plate XXXII, appears to admit of no other explanation than that of conjugation.

The further examination of these and other specimens developed some other peculiarities of interest. Single parasites of each of the above types could apparently be traced through later stages of development. The small, coarse, densely staining body remained comparatively compact throughout its development. It was rarely found in distinct ring form, enclosing hæmoglobin, but often exhibited coarse amœboid processes. It was usually of smaller size than the average tertian parasite, but the infected red cells were swollen and pale. In the full-grown stage this body was compact and densely staining, with rather distinct chromatin granules, but I could not trace it up to a sporulating body (Plate XXXII, Fig. 13). In many respects these large forms resemble the quartan parasite, but the infected red cell is swollen, the pigmentation is not marked and the majority of them, in younger stages, have been found to conjugate with the ordinary tertian rings.

The other type of parasite of the conjugating pair also frequently developed singly, but I am not certain that it reached sporulation. The young forms showed the delicate ring shape with thin bow (Plate XXXII, Figs. 3-5). The larger rings enclosed much hæmoglobin and often exhibited amœboid figures. The infected cells were distinctly swollen and pale. The full-grown form stained very slightly and appeared hyaline, while its chromatin was slight in quantity and minutely subdivided. No presegmenting bodies could be found in these cases which appeared to show the characters of this pale full-grown parasite. All presegmenting bodies and rosettes were either densely staining (before distinct reticulation), or of large size and with abundance of chromatin, the former developing from the compact forms described, the latter apparently from the conjugating parasites but possibly also from the single rings. Possibly the pale hyaline full-grown forms with finely subdivided chromatin were destined to become flagellate forms (compare MacCallum's two varieties

of crescents). Some of the above features are illustrated in the accompanying drawings (Plate XXXII).

Some considerations which do not favor the belief in a process of conjugation require mention:

1. The suggestion naturally arises that the presence of two masses of chromatin does not necessarily mean the presence of two parasites in one red cell.

From a long series of observations on the character of the chromatin in young tertian parasites I must admit that this objection is partly valid. The young tertian parasite, in some cases, may be found to contain two masses of chromatin. In the young compact body (mentioned above) (Plate XXXII, Figs. 1 and 3) these granules when present are large and of nearly equal size, but in the delicate tertian ring I have never seen two distinct and equal chromatin granules. In somewhat rare instances the ring shows an accessory granule of small size in the neighborhood of the main granule, but never, in my observation, have two large granules occurred in a single thin ring-shaped tertian parasite. The significance of these double granules is not clear. The appearance of two, very small, compact, spore-like bodies partly fused together, as may occasionally be seen, indicates that such forms may sometimes result from the early union of the bodies of two very young parasites. The accessory granules in thin ring forms have always appeared too small to have been derived in the same way. It seems probable that such accessory granules may result from the incomplete fusion of the original granules which go to form the chromatin of the spore, or, in other instances, from a precocious subdivision of chromatin in the young parasite, as suggested by Ziemann.

The presence of two nuclei in some very young compact parasites before they enter upon the process of conjugation with the large rings explains the occasional appearance of *three nuclei* about to unite, as seen in some conjugating forms toward the completion of the process (Plate XXXII, Figs. 9 and 10). In one case, the small single compact forms with two nuclei, and large conjugating forms with three nuclei were present in considerable and about equal numbers. That the presence of three, large, entirely separate, subdivided nuclei in

one conjugating form means the union of three original parasites, I do not believe; but the morphological appearances above described indicate that it invariably means the union of at least two parasites.

It appears, therefore, that the presence of two large and equal masses of chromatin in one infected cell indicates, with few exceptions, the presence of two parasites. Rarely three nuclei are seen in conjugating forms, two of which may be derived from two very young compact forms uniting very early, and the third from subsequent conjugation with a thin ring-shaped form.

The further development of young parasites with small accessory chromatin granules may be followed in rare instances. The accessory granule divides as does the main mass of chromatin and later unites with the other to form one clump of granules in the full-grown stage. Throughout these stages the total bulk of these two masses appears not to exceed the average for single parasites, whereas in the conjugating forms the excessive quantity of chromatin in all stages is a very striking feature. In all the examples of such single parasites that I have seen, the unequal size of the chromatin masses was distinct, and there were no appearances suggesting the presence of two parasites in the same cell. These forms, therefore, differ entirely from the conjugating forms above described. I have never seen more than two masses of subdivided granules in a single parasite, whereas three large and equal masses may be observed in conjugating parasites.

A third minute granule may rarely be seen, however, in young rings. Ziemann[1] describes the appearance of multiple chromatin masses in young tertian parasites. He was at first uncertain whether this appearance was referable to the presence of two fused parasites or to an early division of one nucleus, but finally accepted the latter explanation. He describes the separation of one, or rarely two, accessory granules from the original mass in cells infected by single parasites. Sometimes the accessory granule was much smaller than, sometimes nearly as large as, the main granule. All of these appearances I have seen in single parasites, less often in

[1] Ziemann, *Centralbl. f. Bakter.*, 1897, xxi, p. 643.

single members of conjugating pairs, and I agree with Ziemann as to their significance, but the conjugating forms above described are quite different, and do not appear in Ziemann's descriptions.

2. It may be objected, further, that it is impossible to determine when the bodies of two parasites are really united, as one may overlap the other and produce a false appearance of union.

This difficulty is undoubtedly present with some of the young forms, but with others the appearances of the parasites toward the completion of the process, when amœboid motion is subsiding, are, on the contrary, absolutely convincing that the bodies are actually fused. The significance of two large masses of chromatin surrounded by one achromatic zone is also unmistakable (Plate XXXII, Figs. 9 to 11).

3. Again, it may well be pointed out that examples of twin parasites of advanced development, presegmenting bodies and rosettes, are sometimes seen in severe tertian infections, furnishing examples of twinning when conjugation does not occur.

This fact is a matter of common observation, and in my series there are a few cases in which it was especially noted. In one red cell a typical rosette with many spores and a compressed hyaline body without apparent nucleus were observed. In another distended cell were seen one perfect rosette, one imperfect presegmenting body, and one compressed hyaline form.

It may be said of these twins, which proceed to segmentation without conjugating, that they are vastly less numerous than the conjugating forms or young twins seen in the same or other cases. I have, for instance, seen hundreds of conjugating forms within the past few months, but I remember only three or four twinned rosettes seen in as many years.

In the cases showing twinned adult parasites a few younger couples were seen, which showed no attempt to conjugate. The great majority of these young parasites and all the young twins in these cases were the typical tertian *ring-shaped* parasites, while the small compact forms were exceedingly hard to find. My observations on this latter point, however, are not so numerous as is desirable and are still in progress.

4. Finally, the comparative absence of older twinned parasites may be referred to the death and extrusion of one of the twins while the other proceeds to full development alone.

In some gregarines in which multiple infection of cells and conjugation of parasites is common (*Klossia*), one of the parasites often succeeds in dwarfing its companions and alone reaches full development. The dwarfed or dead parasites are then found in the cell alongside the growing form (Wolters, Clarke). In some instances of multiple infection by full-grown or segmenting malarial parasites, I have sometimes seen evidences of compression and death of the younger of two or three organisms. More often both parasites appeared to be equally favored. In any case, the remains of the dwarfed parasite ought frequently to be found if one member of the pair commonly inhibits the growth of the other. In the four cases referred to above, no traces of dwarfed parasites could be found, and while young twins were extremely numerous, all the older parasites were single. It therefore appears impossible to explain the entire absence of older twinned parasites, and especially of traces of any abortive individuals in these cases, on any other ground than that of conjugation.

I find, therefore, that the usual fate of twinning of tertian parasites is conjugation; that twins sometimes grow to maturity without conjugation, for reasons which are not clear, but apparently when both parasites show the usual ring form; that the union sometimes involves three parasites but probably always requires the presence of one or more compact densely staining forms, which do not commonly assume the ring shape, and of one of the typical tertian rings.

A further inquiry relates to the uniformity with which conjugation occurs, and its position as an essential or as an accidental phenomenon in the progress of malarial infection.

It would seem that a process so fundamental as the conjugation of individuals, if it occurs at all, ought to be an invariable feature of every active infection, but there is not sufficient evidence on which to base any such claim. The four cases referred to as furnishing numerous clear examples of conjugation were selected on account of the abundance of the conjugating forms, but in many other cases less

numerous though equally distinct examples were seen, indicating that the process is of very frequent occurrence. On the other hand it must be admitted that the majority of specimens from routine cases fail to show any distinct traces of the process; from which it may be concluded that conjugation is probably not an essential feature of the growth of the parasite.

In the four marked cases the infection was unusually rich, one of them showing more numerous parasites than I had ever seen before in benign tertian infections. One patient had just arrived at Montauk from Cuba, in September, two others were suffering from a first attack, in July, in New York City. In one case there was a prompt relapse a few days after quinine was omitted. It is possible that important clinical features may be found to be associated with the presence of conjugating forms, but the observations are too limited to furnish any conclusion on this point.

In æstivo-autumnal infections, in which twinning is very common, I have been unable to trace the parasites through the conjugating period on account of their disappearance from the peripheral blood. Of five cases showing rosettes and pre-segmenting bodies in the blood, in one were found several twins of these older forms in the same red cell, while in four others no twin parasites were found beyond the ring stage, in which evidences of conjugation, as described by Mannaberg, were occasionally seen. The presence of double nuclei in peculiar æstivo-autumnal rings has been noted. Marchoux suggests that such forms result from conjugation, an explanation which appears reasonable but which is at present without proof. Another more probable explanation has already been mentioned (p. 452).

I have been unable to secure any recent specimens of quartan parasites.

Double rings with fused nuclei are apparently a common form of the young parasite of Texas cattle fever (Theobald Smith).

VIII. ON THE PLURALITY OF SPECIES OF MALARIAL PARASITES.

The belief in a plurality of species of human malarial parasites has been accepted probably by a majority of clinical observers residing in temperate climates, but seems never to have gained uniform sup-

port from those who have studied largely in tropical climates, nor from comparative biologists.

The doctrine of plurality of species is maintained by Mannaberg, Koch, and the great majority of German writers, by Welch, Osler, Councilman, Thayer, Dock, and practically all American writers, and by Golgi, Grassi, Bastianelli, and Bignami, representing the Italian school. A middle ground is held by Kruse, Canalis, Marchiafava, Celli, and Sanfelice, Babes and Georghiu, Danilewsky, and Ziemann (in his early publications), who are inclined to accept the unicist theory, or claim at least that the facts do not warrant the belief in the existence of distinct species; while Laveran, Metchnikoff, Marchoux, Vincent, and some others actively uphold the existence of a single polymorphous species.

Among the pluralists no uniform basis of classification has been established. Those who rely strictly upon the morphology of the human malarial parasites rather uniformly agree upon the existence of three species—quartan, tertian, and æstivo-autumnal. Grassi and Feletti, influenced by the morphology of similar parasites in lower animals, add a fourth distinct species, *Laverania malariæ* (yielding the crescentic bodies), as well as *Hæmamœba immaculata*. Golgi regards the distribution of the parasite in the body of quite as much importance as a ground of classification as its morphology, and therefore makes two groups, one, including the quartan and tertian parasites, which are found principally in the peripheral blood, and a second, the æstivo-autumnal, which is found principally in the internal organs. Mannaberg regards the presence or absence of syzygia, *i. e.*, crescentic bodies, as the chief ground for the separation into species, and recognizes as species which do not produce crescents, (1) the tertian and (2) the quartan parasites, and as those which by conjugation form crescents, (1) the malignant tertian, (2) the pigmented quotidian, and (3) the unpigmented quotidian parasites.

Van der Scheer and Plehn, working extensively in India and Africa, find only two well-distinguished species, (1) the large and (2) the small forms. The former include the quartan and tertian parasites, the latter, the æstivo-autumnal or tropical group of other authors.

It is seen that while there are no distinctly contradictory views among the pluralists, there is an entire lack of agreement in regard to the grounds required for the separation of species. Since there is no room to doubt that a certain stability exists in the three generally recognized species of parasites, practically the question at issue is

whether these species are ever interchangeable, and, if so, to what extent and under what circumstances may one species pass into another. Mannaberg has fully presented the evidence in favor of a plurality of species, without, however, considering many opposing facts, and Laveran has ably supported his own belief in a single species, disregarding much contradictory morphological evidence. While it is unlikely that the question will be fully settled until the extracorporeal form of the parasite has been fully traced, there are some recent observations on the subject which may be profitably reviewed.

The strongest evidence in favor of a plurality of species is found in the results of experiments on the inoculation of malaria, which, when properly controlled, have invariably produced the type of organism found in the specimen of blood used in the inoculation. Mannaberg tabulates 33 experiments of this nature, in 30 of which the inoculation produced the type of organism found in the inoculated blood, while in three the result was doubtful. To these may now be added one case successfully inoculated with the æstivo-autumnal parasite by Zagari and Pace; Sacharoff's infection of himself with the æstivo-autumnal parasite taken from a leech; and two tertian, six æstivo-autumnal, and two mixed infections by tertian and æstivo-autumnal parasites reported by Elting.[1] There are thus at least 42 experiments in which the inoculation of a certain variety of parasite was followed by fever and the growth of the same parasite in the blood. Here must be mentioned also the transference of æstivo-autumnal and tertian infections, successfully accomplished by Bignami, Grassi, and Bastianelli, through the agency of mosquitoes. The invariable reappearance in the infected individual of the type of parasite contained in the injected blood is undeniably strong evidence of permanency of these malarial species. On the other hand, the inoculation experiments by no means prove that the so-called species are immutable *under all conditions*. Accordingly, the passage of the parasite through the bodies of mosquitoes and its reappearance unchanged in subjects thus inoculated by Bignami, Grassi, and Basti-

[1] *Zeitschr. f. Hyg. Med.*, 1899, xxxvi, p. 491.

nelli, must have far higher value as evidence of the immutability of the species. Yet the same element of doubt attaches even to the latter experiments, which may merely indicate that the proper conditions for the transformation of species have not yet been furnished artificially.

A further line of evidence of the same general character is cited by the pluralists in the immutability of species demonstrated in individuals who have been kept under observation for months. It is a matter of common experience that patients who suffer relapses after long intervals sometimes extending over years, usually show the same type of parasite in the relapse as in the initial attack.

Calandruccio examined a triple quartan infection daily for months and found only quartan parasites, and in two cases crescents were found to persist in the blood for two and six months respectively, without the appearance of any other type of organism. Grassi and Feletti also found no change in the type of parasites in a case of quartan fever examined for two months, and in a case of æstivo-autumnal infection examined from October to March.

The permanency of the quartan infections might be expected, but the observations on æstivo-autumnal cases are valuable indications that æstivo-autumnal infections may, at least in some localities, persist unchanged through the winter. In this field, however, the unicists are able to offer cogent evidence in support of their claims. Antolisei (quoted by Bignami and Bastianelli) has seen patients with æstivo-autumnal infection remaining in the hospital all winter manifest tertian paroxysms with tertian parasites in the blood in the spring. They, however, regard these cases as examples of latent tertian infection.

On this same point the observations of Marchoux, during an extensive experience in Senegal, are of interest. He believes that, during the rainy season, in susceptible individuals the cycle of the malarial parasites in Senegal lasts 24 hours at the height of the season, but varies in different cases. In Europeans it tends to shorten and the fever becomes remittent or continuous. In the dry season, the cycle is longer, and the volume of the parasite increases. In many patients with a history of old seizures, who have acquired some immunity, the parasite increases in size and at length, he says, becomes identical with the mild tertian species. During the rainy season when the newly arrived Europeans are suffering from infection with the small ring forms, the native

muleteers, when attacked, all show the large tertian forms in the blood. These observations, while evidently lacking in precision, must be regarded as most significant of the underlying conditions governing the character of malarial infection in the tropics.

Ziemann's[20] experience led him to conclusions very similar to those of Marchoux, viz., that the biological and morphological peculiarities of the parasite may be altered by change of climate and differences in individual susceptibility. Thus in a patient who had recently returned from Kamerun, typical quartan fever was found associated with æstivo-autumnal parasites, while in another instance an ordinary tertian fever was developed. Ziemann also noted the prevalence of irregular fevers with small parasites among Europeans, while the natives were suffering from quartan infections. In his monograph on malaria[21] published in 1898, Ziemann opposes the unicists, and acknowledges himself as a supporter, so far as existing evidence goes, of the doctrine of plurality of species.

My observations at Montauk on cases recently arrived from Cuba, and later in New York City, developed some facts of interest in this connection.

In two cases examined in August large tertian and small æstivo-autumnal ring forms and crescents were found in the blood, but when these cases were examined three weeks later only large tertian parasites were found after a prolonged and repeated search. An irregular administration of quinine had apparently rid the blood of the pernicious type of parasite, leaving the benign tertian form to reappear in the relapse.

Of 335 cases in which parasites were identified in the blood, at Montauk, only 20 per cent (including mixed infections) showed the large tertian organism. But during the past winter (1898-99) I examined the blood of 15 volunteer soldiers who were suffering from relapses of malarial fever contracted in Cuba, and all showed the large tertian parasite only. This same experience has been the rule at various hospitals and dispensaries of this city. In one of these patients the blood was examined in August by a competent observer and found to contain æstivo-autumnal rings and crescents, but in Jan-

[20] *Centralbl. f. Bakter*, 1896. xx, p. 653.

[21] Ueber Malaria- und andere Blutparasiten.

uary only the tertian parasite could be found during a sharp relapse. It is of course possible that mixed infection existed in this case, but there is no clear explanation of the failure to find the tertian parasite in August. Also, to reconcile the fact that the tertian parasite was always found in the above-mentioned relapses with the theory of immutable species requires considerable straining of the facts known in regard to the relative virulence of the species and their relative susceptibility to quinine. It appears more reasonable to suppose that under the influence of cold weather, and gradually increasing systemic resistance, the æstivo-autumnal parasite was replaced by the tertian in the relapses suffered by these volunteers. Or it may be suggested that the cold weather alone stamped out the æstivo-autumnal infections, leaving only the tertian cases to relapse during the winter.

The disappearance of the æstivo-autumnal infections during the late autumn and winter recalls the fact that the distribution of the types of infection is determined by climatic conditions, although, as Laveran puts it, "if the species are separate, there ought to be geographical foci where tertian or æstivo-autumnal infections largely predominate, whereas all forms of malaria are commonly contracted wherever malaria is endemic."

The theory of "mixed infections" also has been made to bear a heavy burden in order to support the belief in separate species. In the ordinary type of mixed infection the tertian amœboid forms are associated with crescents, but one rarely finds both amœboid forms, with or without crescents, in the same individual. Yet if the patient is susceptible to malaria why should he retain the mild tertian amœba, while the small malignant forms disappear?

The lack of permanency observed in the mixed infections is also a suspicious feature of the condition. Two types of parasites seldom remain long together in the same subject, one very shortly displacing the other, as shown both by clinical observation and in experimental infections (cf. De Mattei, Calandruccio). As a rule, it is the more highly vegetative tertian parasite which in clinical experience displaces the more malignant æstivo-autumnal, but this rule may be reversed in experimental infections. De Mattei saw an old quartan

infection disappear after experimental infection by the æstivo-autumnal parasite, as well as the disappearance of æstivo-autumnal parasites after inoculation with quartan. Gualdi and Antolisei record two cases of quartan infection seen in May, which showed æstivo-autumnal parasites in the autumn.

The frequency of mixed infections is undoubtedly an argument against the plurality of species, showing that there is a very close connection between the sources of the tertian and æstivo-autumnal forms of parasite. In my preliminary report of the Montauk cases there were noted 12 examples of double infection out of 86 tertian cases observed. By a subsequent review of a minority of these specimens the number of mixed infections has been nearly doubled by the discovery of single crescents in tertian cases, or of single large tertian organisms among many crescents. I believe that mixed infections are much more common in the severe cases of the tropics than present reports indicate, and that their recognition depends largely on the time one cares to spend on the examination of the blood.

All the above difficulties may, however, be adjusted to the theory of a plurality of species, and in the absence of more definite knowledge of the extracorporeal form and development of the parasite it is unlikely that the question can be settled on such general considerations as those adduced.

Turning to the comparative morphology of the parasite, the evidence both for and against the plurality of species becomes much more specific. Here the pluralist doctrine finds its chief support and, whatever may be the final outcome of the discussion, it cannot be doubted that the three groups of parasites, quartan, tertian, and æstivo-autumnal, exhibit morphological characters which are to a large extent immutable. Yet the two widely different forms—the æstivo-autumnal rings and the crescents—are regarded as belonging to the same species, and the whole groundwork of a morphological classification is found to be insecure on account of the extreme polymorphism observed throughout the entire group of protozoa. Of this a few details may here be briefly considered.

One of the most striking differences between the tertian and the æstivo-autumnal parasites is the dissimilarity in the staining quality of

their nuclei. Methylene blue stains the nucleus of the young æstivo-autumnal ring densely but fails to stain the nucleus of the tertian ring. This difference probably depends upon an unequal mixture of oxy-chromatin and basi-chromatin in these nuclei. I cannot find that similar differences are recognized in the staining qualities of any two species of coccidia or of gregarines, but somewhat similar differences were noted between different phases of the same species of coccidia by J. Clarke, and by Siedlecki.

The study of flagellated bodies in related protozoa may be found to bear on the question of a plurality of species of malarial parasites. In *Coccidium oviforme* flagella have been found to develop from large spheroidal bodies. This protozoan produces crescents, but these have not been traced through exflagellation (Simond). In *Benedenia ovata* flagella have been found to develop from large spheroidal bodies. This protozoan also produces crescents, but these have not been traced to a flagellating stage. In *Adelea ovata*, on the other hand, the exflagellation of crescents has been observed, but large spheroidal forms producing crescents have not been described (Siedlecki).

It would appear that most coccidia and gregarines produce flagella both from large spheroidal bodies and from crescentic forms. Although the homologues of these forms in the malarial parasites are not fully determined, this fact, if fully established, would strongly indicate that the tertian malarial parasite producing flagella from large spheroidal bodies, and the æstivo-autumnal parasite, with flagellating crescents, are phases of one and the same protozoan.

It may be claimed that if the so-called malarial species are interchangeable, transitional forms ought to be abundantly present in some cases, but these have not been fully demonstrated. Yet it appears by no means certain that in order to establish the unity of the malarial parasite it is necessary to assume the existence of transitional forms. In *Benedenia octopiana*, the formation of crescents is preceded by an entire bisexual cycle of development in which the male element is furnished by large spheroidal flagellate bodies. The intermediate forms of the two cycles in this protozoan differ considerably from each other, there are no transitional forms between them, and yet both belong to the same parasite.

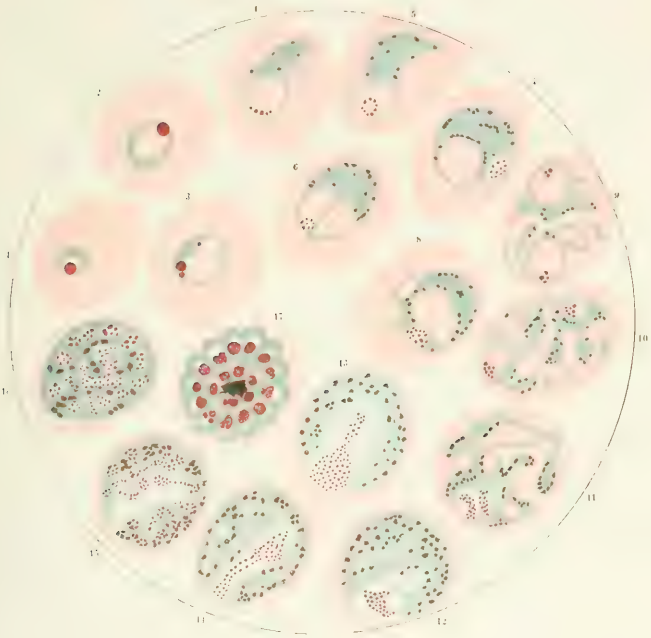
While it is true that no transitional forms between the æstivo-autumnal and the tertian parasite and between the tertian and the quartan parasites have been fully described, there are numerous observations indicating that such forms exist. The slight differences

in size, refractive quality and amœboid activity which led Marchiafava and Bignami to separate a quotidian from a tertian æstivo-autumnal species, a position from which they have largely receded, have been noticed and regarded by others as occasional differences in the morphology of one æstivo-autumnal species (Ziemann, Gautier, and others). Marchoux claims to have observed a gradual increase in the size of the æstivo-autumnal parasite during the healthier seasons in Senegal. When one closely examines the parasites seen in the average tertian case of this climate, isolated forms may be found, in greater or less numbers, which closely resemble the quartan parasite. The red cells are not always swollen when infected by tertian organisms, and these parasites are sometimes compact and very richly and coarsely pigmented at an early stage. Of the two somewhat distinct forms of parasites which I find commonly enter into the conjugating pairs of the tertian series, one is compact, of rather small size, and resembles the quartan parasite in some particulars, but the infected cells are swollen.

In examining the blood of volunteer soldiers who were suffering during the past winter from relapses of malarial fever contracted in Cuba, I was early struck with the resemblance which many of the young tertian parasites bore to the young æstivo-autumnal rings. In some of these cases the young tertian rings closely resembled the young signet-ring form of the æstivo-autumnal parasite, exhibiting a very thin bow and a distinct circumscribed swelling of one segment. Their chromatin, moreover, was often found subdivided before the appearance of pigment, although in the vast majority of mild tertian cases seen in New York City, the chromatin of the young tertian parasite is not subdivided till after pigment appears (cf. Gautier). The nuclei of these forms usually failed to stain with methylene blue, but not a few examples were found among the young rings in which the chromatin stained well with methylene blue. The usual swelling of the infected cell was often very slight, or sometimes absent, in these cases. It appeared quite possible to trace the development of these young rings up to the larger amœboid stage when the tertian characteristics become distinct. No crescents were found in any of these cases.

Several explanations may be offered to account for these peculiarities. It may be supposed that the young tertian rings do not necessarily differ from the æstivo-autumnal. This explanation I am unable to accept, finding that the tertian ring, as occurring in New York, invariably differs from the æstivo-autumnal form imported from Cuba, especially in regard to the staining quality of its nucleus (see p. 437). Or it may be supposed that the cases in question were really examples of mixed infection. Yet the suspicious young rings could be traced in development to the large tertian forms, and no crescents were found in any of these cases. Or, finally, it may be supposed that the peculiarities of the young parasites in these cases represented transitional phases between the æstivo-autumnal and tertian parasites. This explanation I am inclined to accept. The observation of 15 cases in which such peculiarities were noted is, however, insufficient to be convincing, and satisfactory grounds for the acceptance of such a belief cannot well be furnished except by demonstrating the complete transformation in the same individual of an æstivo-autumnal infection during the winter through various transitional phases in the morphology of the parasite.

Whichever theory may finally be established regarding the varieties of the human malarial parasite, the evidence would seem to justify the opinion of Kruse, Canalis, Babes, Celli, Danilewsky, and others, who regard the existence of several species as not yet proven, and who find not only in malarial parasitology, but especially from comparative biology, that the phenomena of the disease are more easily reconciled with the existence of a single polymorphous species. Certainly, in many ways, the knowledge of the disease would be furthered by adherence to the unicist theory as a practical working basis.



Developmental Cycle of Benign Tertian Parasite.

FIG. 1. Very early form of parasite, showing chromatin granule, "milky zone," and spheroidal body.

FIGS. 2, 3. Typical young ring-shaped parasites.

FIGS 4, 5. Subdivision of chromatin, development of body and appearance of pigment in later ring-forms.

FIG. 6. Double rings, in single parasite.

FIGS. 7, 8. Earlier stages of parasites. Secondary rings. Eccentric position of chromatin.

FIG. 9. Double infection of cell.

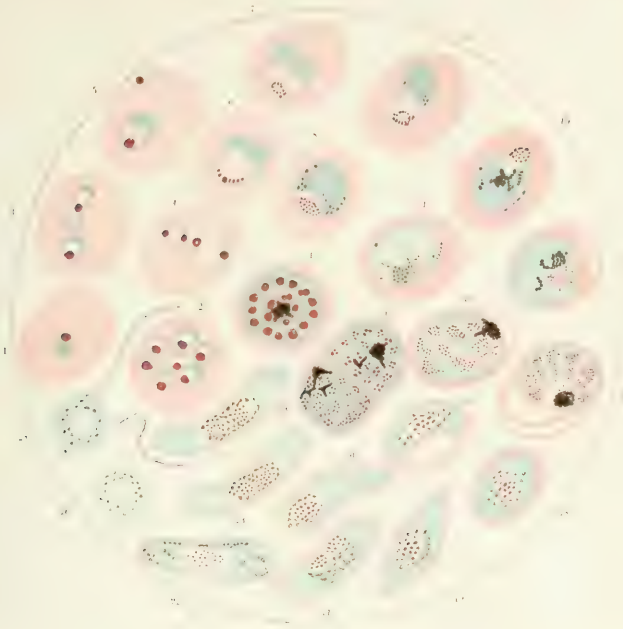
FIGS. 10, 11. Complex amœboid figures in doubly infected cells.

FIG. 12. Full grown form, with large eccentric nucleus.

FIGS. 13, 14. Protrusion of chromatin granules and milky substance in body of full-grown parasite.

FIGS. 15, 16. Division of chromatin granules into groups at right angles to pigmented segmenting bodies.

FIG. 17. Tertian rosette.



Cycles of Æstivo-autumnal Parasite.

[illegible]

FIG. 2. Infection of one cell with seven young parasites. (Drawn from a marrow-smear.)

FIG. 3. Triple infection. Two parasites joined by single chromatin mass.

FIG. 4. Double infection. Peculiar rings with two chromatin grains at opposite poles.

FIG. 5. Double infection. Small ring adherent to cell.

FIGS. 6, 7. Signet-ring forms. Sub-division of chromatin.

FIGS. 8, 9. Later ring forms, with sub-divided chromatin and few pigment grains.

FIGS. 10–12. Full-grown forms with finely sub-divided chromatin and gradual concentration of pigment.

FIGS. 13, 14. Stages of presegmenting forms, with concentrated eccentric pigment.

FIG. 15. Double infection with separate presegmenting bodies.

FIG. 16. Æstivo-autumnal rosette.

FIGS. 17, 18. Young crescent and ovoid.

FIG. 19. "Pulsating" crescent.

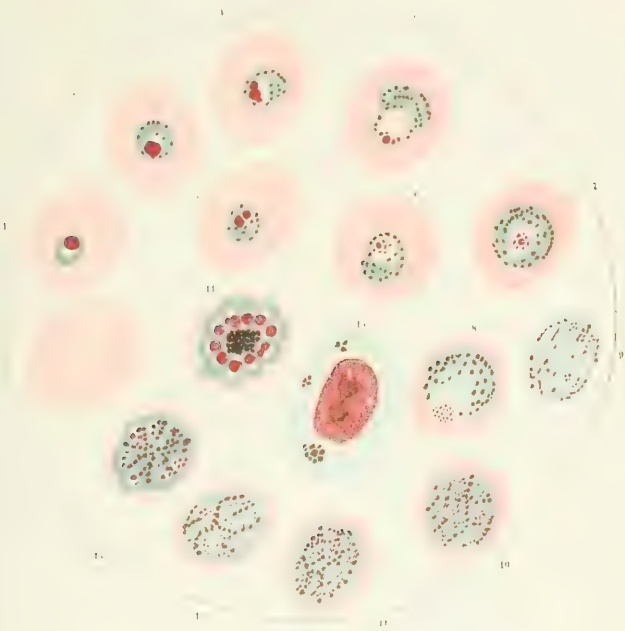
FIGS. 20–22. Various forms of crescents.

[illegible]

FIG. 24. Finely developed crescent; two masses of chromatin; achromatic substance; double wreaths of pigment.

FIG. 25. Diagrammatic flagellating body.

FIG. 26. Extra-cellular sterile body.



Cycle of Quartan Parasite.

Figs. 1 [illegible] Young [illegible] Plasmodium.

Figs. 2, 3, 4. Small [illegible] rings, with large chromatin [illegible] and [illegible] of pigment.

Fig. 5. Turban-shaped ring, with subdivided chromatin.

Fig. 6. Subdivision of ring and of chromatin granules.

Fig. 7. Coarse quartan ring with central chromatin granules.

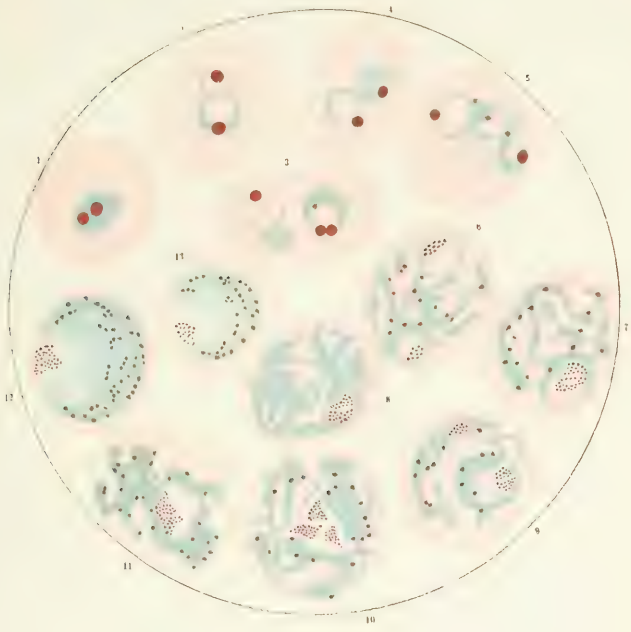
Fig. 8. Full-grown quartan parasite, with eccentric chromatin, hyaline body, and abundance of pigment.

Fig. 9. Extra-cellular reticulated body.

Figs. 10 [illegible] Segmenting [illegible] forms.

Fig. 14. Quartan rosette.

Fig. 15. Pigmented mononuclear leucocyte.



Conjugating Cycle of Tertian Malarial Parasite.

FIG. 1. Single compact body with double chromatin masses.

FIG. 2. Conjugating rings of unequal size.

FIG. 3. Double infection with a coarse ring, double chromatin granules, and a thin ring form.

FIGS. 4, 5. Early stages of conjugation of a thin ring and a compact body.

FIG. 6. Early amœboid figures of conjugating rings.

FIG. 7. Double nuclei in amœboid parasite.

FIGS. 8, 9. Union of nuclei, and subsidence of amœboid motion in older conjugating parasites.

FIGS. 9, 10. Stages of union of bodies and of three chromatin masses, of two conjugating parasites.

FIG. 11. Complete union of bodies and nuclei.

FIGS. 12, 13. Comparative sizes of full-grown forms developed with and without conjugation.

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THE WESLEY M. CARPENTER LECTURE ON CONJUGATION IN THE ASEXUAL CYCLE OF THE TERTIAN MALARIAL PARASITE.*

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IN 1897 the writer's attention was attracted by a specimen of blood from a rich tertian infection in which there were very numerous twinned parasites of about twelve hours' development, while all the older parasites were single. A remarkable specimen of this type was secured at Montauk, in 1898, from a soldier just arrived from Cuba, in whose blood there were two very compact and very numerous broods, nearly all the young parasites being twinned while all the older forms were single. Extreme length and variety of amœboid processes characterized the younger parasites in both cases. The latter case promptly raised a suspicion that under some conditions young tertian twins might conjugate, producing a single large full-grown parasite.

In the following spring two pronounced cases of the same type were observed in New York. In one of these the writer was able to follow the parasites through one complete cycle, and found that the young twins were succeeded by single full-grown parasite. At the same time, by the application of the Nocht-Romanowsky method of staining the parasites, all stages of the gradual union of nuclei were clearly demonstrable, and the writer's suspicion regarding a possible conjugation of young parasites reached a positive conviction.

Since that time other cases have been observed which have supported the conclusions reached in 1899, and presented in a preliminary report at Baltimore in March, 1900.

The evidence favoring the belief in conjugation of parasites consists principally in the morphological appearance of twinned

*Delivered at the New York Academy of Medicine, November 15, 1900.

organisms in their developmental stages. The blood in typical cases usually shows two broods, the younger consisting of twinned compact and ring-shaped parasites, and the older of single large or full grown parasites. In many instances the young organisms are entirely separate, each exhibiting a single large granule of chromatin. Many red cells, however, contain two young parasites which are clearly fused together along one segment of their bodies, and two large chromatin granules are then invariably found at distant points in the parasites.

The fused bodies usually differ in appearance. One is then a large, delicate ring with thin bow and chromatin granule of moderate size, while the other is a coarser body, often compact, or, if ring-shaped, exhibiting a thicker bow, inclosing little or no hæmoglobin, and presenting a larger chromatin granule. These differences between the conjugating parasites are not always clearly visible.

Among the rings which occur singly in such specimens of blood, the two forms of young parasites are often distinguishable, but the writer has been unable to find recorded any observation of single rings containing two large and equal chromatin granules. Every red cell containing two large and equal chromatin granules always contains two distinct young parasites.

It appears, therefore, that the bodies of these young twinned parasites become fused together while their nuclei remain separate. Occasionally the two chromatin granules are found close together, but no signs of fusion of nuclei are found at this early stage.

In a somewhat later stage of development most of the parasites are found to have lost the simple ring form and to have developed a number of long, beaded, thread-like pseudopodia, variously curled in the red cells, indicating a very active amœboid stage. The chromatin masses are now subdivided into ten or twelve granules, but these masses still remain far apart and show no tendency to unite.

In still later stages the amœboid figures become less marked and the masses of chromatin lie side by side united by a little achromatic "milky" substance. Finally, the groups of chromatin granules come to lie side by side surrounded by achromatic substance, and this phase is marked by a distinct reduction in the length of amœboid figures. Beyond this stage the parasites show complete fusion of both bodies and nuclei, the resulting individual being of large size and showing an abundance of chromatin.

The morphological evidence indicating conjugation is therefore apparently complete.

There is also the strong circumstantial proof to be mentioned that very numerous young twinned parasites are succeeded by older single forms, and equally significant is it that a full-grown brood, all single, is found associated with a numerous young brood mostly twinned. It is not at all likely that twinning can occur extensively in a part of a brood and not at all in its older members, or in one brood extensively and not at all in its predecessor. The occurrence in the Montauk patient of two broods, one less than half grown and almost invariably twinned and the other approaching segmentation and all single, convinced the writer at once that conjugation was the only possible explanation applicable to the case.

Some possible objections to the theory of conjugation must be considered.

The suggestion naturally arises that the presence of two masses of chromatin does not necessarily mean the presence of two parasites in one red cell. From a long series of observations on the character of the chromatin in young tertian parasites, the writer must admit that this claim is partially valid. The young tertian parasite, in some cases, may contain two granules of chromatin. In the young compact body previously described, these granules, when present, may be large and of nearly equal size, but in the delicate tertian ring the writer has never seen two equally large chromatin granules. In somewhat rare instances the thin ring shows an accessory granule of small size in the neighborhood of the main granule, but never, in the writer's observation, have two large granules occurred in a single, thin, ring-shaped tertian parasite.

The significance of these double granules is not clear. The appearance of two very small, compact, spore-like bodies, partly fused together, as may occasionally be seen, indicates that such forms may sometimes result from the early union of the bodies of two very young parasites. The accessory granules in the thin rings have always appeared too small to have been derived in the same way, and probably result from the precocious subdivision of the main granule. The accessory granules may arise, therefore, (1) from the incomplete fusion of the original granules which go to make up the spore in the parent rosette, or (2) from a precocious subdivision of the chromatin, as suggested by Ziemann, or (3), as seems most likely to be the case with the compact bodies, by the incomplete separation of the bodies of two spores in the rosette.

In any case, the existence of more than one granule in a single parasite presents no difficulty for the theory of conjugation. The identification of twin parasites in the writer's specimens has always been based, not merely on the presence of multiple nuclei, but also on the undoubted presence of the bodies of two distinct parasites. In some of the conjugating forms, moreover, three masses of chromatin granules were rather frequently seen in various stages of union, when only two bodies of parasites were to be found in the red cells. This appearance, which was at first very puzzling, can be positively referred to the conjugation of one compact parasite with double chromatin granules and one thin ring-form with single granule. In one case single compact parasites with double granules twinned with thin ring-forms, and large conjugating forms with three masses of chromatin, were present in considerable and nearly equal numbers. It appears, therefore, that the presence of two large and equal masses of chromatin in one infected red cell indicates, with few exceptions, the presence of two parasites. The exceptions are disposed of by the fact that three nuclei are sometimes seen in conjugating forms, two of which are usually derived from two very young compact forms uniting very early, probably in the rosette, and the third from subsequent conjugation with a ring-shaped parasite.

The further development of single young parasites with accessory granules may be followed in rare instances. The accessory granule divides, as does the main mass of chromatin, and, later, unites to form one clump of granules in the full-grown stage. Throughout these stages the total bulk of the two chromatin masses appears not to exceed the average of single parasites, whereas in the conjugating forms the excessive quantity of chromatin in all stages is a very striking feature.

In all the examples of such single parasites that the writer has seen, the unequal size of the chromatin masses was distinct when there was no indication of the presence of the bodies of two parasites in the same cell. These forms, therefore, differ entirely from the conjugating forms above described, in which multiple chromatin masses are always associated with multiple parasites. The writer has never seen more than two masses of subdivided granules in a single parasite, whereas three large and equal masses may be observed in two conjugating forms.

Ziemann describes the appearance of multiple chromatin masses in young tertian parasites. He was at first uncertain whether these

granules were referable to the presence of two fused parasites or to early division of one nucleus, but finally accepted the latter explanation. He describes the separation of one, or rarely two, accessory granules from the original mass in cells infected by single young parasites. Sometimes the accessory granule was much smaller than, sometimes nearly as large as, the parent granule. All these appearances the writer has seen in single parasites, less often in single members of the conjugating pair; and he agrees with Ziemann that they may represent merely a precocious subdivision of chromatin, but the conjugating forms of the writer's observations are quite different and do not appear in Ziemann's descriptions.

Secondly, it may be objected that it is impossible to determine when the bodies of two parasites are really united, as one may overlap the other and produce a false impression of union. This difficulty is undoubtedly present with some of the young forms; but, with others, the appearance of the parasites toward the completion of the process, when amœboid motion is subsiding, is, on the contrary, absolutely convincing that the bodies are actually fused. The significance of two or three large masses of chromatin surrounded by one achromatic zone is also unmistakable.

Thirdly, it may well be pointed out that examples of twin parasites of advanced development, presegmenting bodies and rosettes, are sometimes seen in severe tertian infections, furnishing examples of twinning without subsequent conjugation. This fact is a matter of rather common observation, and, in the writer's experience, there have been some cases in which it was especially noted. In one infected cell were found, for instance, a large rosette and a compressed hyaline body. In another were seen one perfect rosette, one imperfect presegmenting body, and one compressed hyaline body.

It may be said of these twins which proceed to segmentation without conjugating, that they are vastly less numerous than the conjugating forms of young twins seen in the same or other specimens. The writer has seen hundreds of conjugating forms within a few months, but remembers only three or four twinned rosettes seen in as many years.

In the cases showing twinned adult parasites, a few younger couples were seen which showed no attempt to conjugate. The great majority of these young parasites were the typical tertian ring-shaped parasites, while the small compact forms were hard to find. There is in this fact an indication that conjugation requires the presence of two somewhat differentiated parasites, as already de-

scribed, one a compact body and the other a ring-shaped parasite. Observation of recent cases has borne out this conclusion.

Finally, the comparative absence of older twinned parasites may be referred to the death and extrusion of one of the twins while the other proceeds to full development alone.

In some gregarines in which multiple infection of cells and conjugation of parasites is common (Klossia), one of the parasites usually succeeds in dwarfing its companions and reaches full development alone. The dwarfed or dead parasites are then found in the cell alongside of the growing form (Wolters; Clarcke). In some instances of multiple infection by full-grown malarial parasites there are evidences of compression and death of the younger of two or three organisms. More often both parasites appear to be equally favored. In any case, the remains of the dwarfed parasite ought to be found if one member of the pair commonly inhibits the growth of the other. In the writer's cases showing conjugation no traces of dwarfed parasites could be found, and, while young twins were extremely common, all the older parasites were single. It therefore appears to be impossible to explain the entire absence of older twinned forms, and especially of traces of any abortive individuals, on any other basis than that of conjugation.

The writer finds, therefore, that the usual result of twinning of tertian parasites is conjugation; that twins sometimes grow to maturity without conjugating, for reasons which are not clear, but apparently when both parasites show the usual ringform; that the union sometimes involves three parasites, but probably always requires the presence of one or more compact, densely staining forms, which do not commonly assume the ring shape, and one of the typical tertian rings.

A further inquiry relates to the frequency with which conjugation occurs and its position as an essential or accidental phenomenon in the progress of malarial infection.

It would seem that a process so fundamental as the conjugation of individuals, if it occurs at all, ought to be an invariable feature of every active infection, but it must be admitted that the majority of specimens taken from routine cases fail to show distinct traces of the process. Nevertheless, it is probably a very common occurrence, since the writer has found little difficulty in securing specimens containing a few conjugating forms.

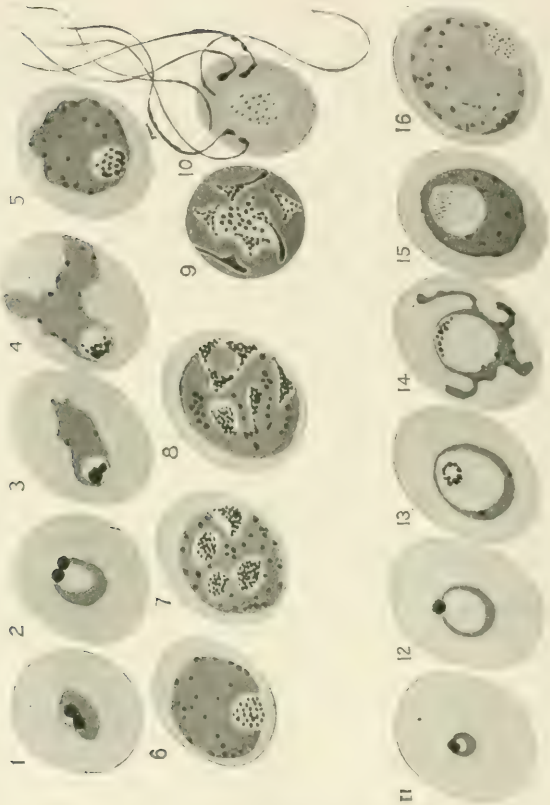
The four cases originally referred to as exhibiting very abundant conjugating forms presented several characteristics in common.

They were all very rich infections, the Montauk case showing more parasites than the writer has ever seen in any other benign tertian infection. The three New York cases were examined early in the course of the disease after severe febrile paroxysms. They all responded promptly to quinine, although one relapsed when quinine was omitted. Conjugating forms are usually few or wanting when the total number of parasites is small, or after quinine has been given, or in third or fourth paroxysms. *Conjugation seems to be a feature, therefore, of the early stages of rich infections.*

This conclusion from clinical data accords with the well-known significance of conjugation among many protozoa, which are able to reproduce two or three generations through conjugation, after which another type of sexual reproduction becomes obligatory.

The further examination of specimens exhibiting conjugating forms develops other peculiarities of great interest.

Single parasites of both types, *i. e.,* compact, densely staining bodies with one or two large chromatin granules, and the thin ring-shaped parasites, can be traced through later stages of development. The compact bodies remain densely staining and compact throughout their growth. They are not often found in distinct ring-form inclosing hæmoglobin, but usually remain compact, while developing short amœboid processes. They are usually of smaller size in all stages than are other equivalent tertian parasites, but the infected cells are slightly swollen. In some respects they resemble quartan parasites. In the full-grown stage these bodies are still densely staining, with very distinct chromatin granules. The writer is not certain that they ever segment, but he has been able to trace them up to a form in which there are six subdivided groups of chromatin granules. (See cuts.) Now, the true presegmenting tertian form does not develop its sixteen to twenty separate nuclei by the subdivision of the parent group of granules first into two, then into four, eight, and sixteen masses, but the whole parent mass flows out into the body and is *at once disseminated* into the final number of nuclei destined to form spores. These densely staining bodies with from four to six chromatin masses are distinctly different in appearance from the true presegmenting bodies. The writer finds them, on the other hand, much more like the bodies which in shed blood exhibit undoubted evidence of the beginning formation of flagella. Indeed, in some of the densely staining compact bodies with six chromatin masses obtained directly from the circulating blood, the chromatin has appeared in elongated rod-form as it appears in forming flag-



DESCRIPTION OF CUTS.

Figs. 1-8 inclusive represent the forms which belong to the development cycle of the compact tertian parasite.

Figs. 9-10 represent phases of formation of flagella in shed blood.

Figs. 11-16 represent the forms which belong to the cycle of the thin ring-shaped tertian parasite.

ella in shed blood. The writer has not traced the extrusion of flagella from these compact bodies with six nuclei, but in specimens made from fresh blood which has been allowed to stand in a moist chamber for from ten to twenty minutes and stained by Nocht's method, flagellating forms show marked resemblance to the compact bodies with six nuclei seen in specimens made from fresh blood in the ordinary way, but those flagellating forms do not stain so densely with methylene-blue.

Likewise the development of single ring-shaped tertian parasites may be traced in the same specimens with the others. As these forms increase in size they are characterized by their hyaline appearance and tendency to stain lightly with methylene-blue, and by the comparatively small quantity of finely divided chromatin. No presegmenting bodies could be found in these cases which appeared to show the characters of this pale full-grown parasite, and the writer is not certain that it ever segments. On the other hand, the pale-staining, often extra-cellular, non-flagellating bodies seen in blood from fifteen to twenty minutes after shedding show considerable resemblance to the pale forms derived from single ring-shaped parasites which have not conjugated.

These observations accord in some, but not in all, respects with the descriptions given by Bastianelli and Bignami of the tertian microgametocytes and macrogametocytes in the mosquito's stomach.

According to these observers, the microgametocyte, or male flagellating form, exhibits from five to six times as much chromatin as the other. This chromatin may lie in one central mass or in a network of threads, but is always compact and densely staining. It nearly always produces six flaggella. The macrogametocyte, or female form, shows an eccentric nucleus with very scarce chromatin granules, and is a very pale-staining body. They trace these two forms indistinctly in the circulating blood, as follows:

"From nine to six hours before the chill, besides tertian rosettes, there are to be seen bodies of equal or larger size, with a single vesicular nucleus and subdivided chromatin grains or rods which lie far apart. In bodies one-third smaller than the above-described forms similar rods and threads may be seen, and it is reasonable to suppose that the forms destined for development in the mosquito have from their earliest stages a characteristic form. In the circulating blood, however, these early forms are not seen, because, as in the development of crescents, they are confined to the marrow."

The writer has not had opportunity to examine malarial blood from the mosquito's stomach, but in shed blood kept moist for fifteen minutes he has seen the early stages of the formation of flagella, and finds such bodies to resemble in size, number of chromatin masses, and general staining qualities the fully developed compact form seen in conjugating specimens.

That microgametocytes and macrogametocytes develop especially in the bone marrow, as suggested by Bastianelli and Bignami, may explain their usual absence from the peripheral blood, where the writer has seen the separate development of the two distinct forms of parasites in only a very few cases. At present, analogy with the æstivo autumnal parasite is the only evidence favoring the suggestion that the bisexual forms of the tertian parasite develop principally in the bone-marrow, as the opportunity to examine the marrow of cases of tertian malaria is, fortunately, very rare.

In the æstivo-autumnal parasites, the bisexual forms, the crescents, have for some time been separated into two classes. McCallum describes distinct differences between the flagellating crescent, or male microgametocyte, and the non-flagellating female macrogametocyte in the parasites of birds. Bignami and Bastianelli, as also Celli, note several distinguishing points in the male and female crescents of the human parasite. According to these observers the microgametocyte is smaller, assumes the spheroidal form, stains lightly with methylene-blue, and its nucleus is apt to be eccentric and not to show a vesicular type. The macrogametocyte retains the fusiform shape, stains densely, and exhibits a central vesicular nucleus surrounded by pigment. After fertilization, the macrogametocyte stains still more densely, the nucleus may migrate to one pole, and the pigment becomes disseminated. All these features the writer has found to characterize individual crescents in circulating blood, and cannot regard them as indications of fertilization unless it is admitted that under some conditions crescents may become fertilized in the circulating blood.

Mannaberg believes that crescents are syzygia developed by conjugation of two amœboid individuals. Recent study of related protozoa, however, has left little support to such an hypothesis. Crescentic bodies in protozoa invariably develop by segmentation of an encysted body, and the writer can find no instance of the development of a single sex-ripe form by conjugation of two individuals. At any rate, if crescents develop through conjugation, the process

is of entirely different significance from the present type of conjugation of tertian parasites.

A natural inquiry relates to the occurrence of conjugation among æstivo-autumnal parasites, with which twinning of young forms is decidedly more common than with the vernal parasite.

An increased difficulty in determining this question is found in the disappearance of æstivo-autumnal parasites from the peripheral blood just at the time when conjugation of forms might be expected to occur. The writer has already described two forms of æstivo-autumnal rings, but has been unable to trace their development, either in smears of the viscera of fatal cases or in the peripheral blood of five cases in which numerous parasites of all stages remained in the peripheral blood. During this search for conjugating forms, several examples of twinned, half or full grown, or segmenting, parasites in the same red cell were encountered, but they were vastly less numerous than young twins. If conjugation occurs it probably lasts over a very brief period only, and can be demonstrated only in smears of visceral blood where the parasites have massed in large numbers. In a few quartan specimens the writer has seen no traces of either twinning or conjugation.

The Biological Significance of Conjugation.—Comparative biology offers an abundant opportunity for the occurrence of conjugation of malarial parasites. Of protozoa, conjugation is most frequently observed among gregarines, which exhibit at least two types of the process. In one of these types, one or more embryonal gregarines attach themselves to the body of a second or third individual, forming a short chain, much as do certain bacteria (Klossia). In this relation they proceed for a variable time in their development. Pfeiffer refers to this process, apparently, as a "spurious conjugation," employing the term "embryonal cohesion" (*embryonale Verklebung*). In the second and more important type, two partly or fully developed individuals become encysted together, developing sporocytes and initiating a completely new phase of existence in the life of the parasite.

Pfeiffer describes the gradual fusion, first of the bodies, later of the nuclei, of conjugating sporozoa. Wittaczil also describes the fusion, first of the bodies, later of the nuclei, of a rare form of gregarine, and refers to similar processes in other protozoa. Wolters gives minute descriptions of conjugation in gregarines, in which complex nuclear changes are concerned. In all these cases, however, conjugation precedes encystment, showing that this particular

cycle of the parasite undergoing conjugation is not homologous with the pyrogenous cycle of the malarial parasite, which becomes encysted in the mosquito's stomach after fertilization by the flagellum, and not after conjugation.

On the other hand, it is a well-recognized biological principle, stated, among recent authors, by Leuckart, Pfeiffer, Sedgwick, Haeckel, and others, that conjugation among protozoa may be the only sexual feature of reproduction during a limited number of generations, after which the reproductive capacity ceases and must be restored by the development of bisexual forms, and usually by the appearance of an entirely new phase of existence. With the malarial parasite, conjugation appears to fulfill just such a purpose, favoring the development of the ameboid pyrogenous forms of the organism over a few generations, after which there is a tendency to develop separate bisexual forms, which, in the mosquito's stomach, initiate a new phase of existence in a new host. In this respect malarial conjugation must be admitted to have the full significance which attaches to it in related protozoa, and cannot be classed as a form of embryonal cohesion or of spurious conjugation.

The spontaneous disappearance of malarial infections in the human subject may thus be explained by the gradual failure to reproduce by conjugation, and the development of parasites singly into sexual individuals, sterile for the human host, but adapted to development in the mosquito. Such quiescent stages of related infections are very commonly observed in the blood of snakes and birds, etc., which, for months, harbor with impunity countless numbers of the crescentic bodies awaiting transfer to a new host.

In current literature on related protozoa there appears to be no reference to conjugation of individuals belonging to a cycle which is homologous with the pyrogenous cycle of the malarial parasite. This is probably owing to the fact that observed forms of the coccidia and gregarines belong to the encysted cycle, which in the malarial parasite occurs in the mosquito, while the other forms, if they exist, have not yet been described or fully studied.

The occurrence of conjugation among malarial parasites establishes more fully the parallelism believed to exist between the life histories of this and related protozoa.

The malarial parasite may be said to begin its existence, in the human host, by a series of generations of ameboid bodies produced after conjugation of partially differentiated individuals. After the capacities of this method of reproduction have been exhausted, the

sexes become separated, the male forms being represented by the flagellating bodies, microgametocytes, and the female by the large, pale, hyaline, non-flagellating bodies (macrogametocytes). These forms are sterile for the human host, but in the mosquito fertilization of the macrogametocyte by the flagellum or microgamete takes place, and the resulting motile form, the vermiculus, penetrates the tissues of the mosquito and becomes encysted. From this cyst develop the germinal rods which are inoculated into the human host through the salivary glands of the mosquito, to begin anew the pyrogenous or amœboid cycle. At several points in the series there remain wide gaps in our knowledge. We do not yet know how the germinal rods are transformed into amœboid bodies. The development of the sexual forms of the tertian parasite, as described by the writer, can at present only be offered as a probability, not as a demonstrated fact, while the origin of æstivo-autumnal crescents is at yet entirely obscure. At the other end of the series there is possibly an entire new phase of development of the parasite or the production of some "resistant body" in the external world apart from either man or mosquito. The present paper need not, however, be burdened with the obscurity of these related problems, as its object is merely to present the evidence, which is believed to be demonstrative, of the occurrence and significance of malarial conjugation.

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Extracted from The American Journal of the Medical Sciences, October, 1901.

A CASE OF MALARIAL NEPHRITIS, WITH MASSING OF PARASITES IN THE KIDNEY.[1]

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The frequent occurrence of renal lesions in malarial fever was described long before the discovery of the malarial parasite, and has been very constantly recognized throughout the progress of our knowledge of the disease.

Owing to the studies of Rempicci in Italy and of Thayer in America it is unnecessary to again review in detail the contributions of the various observers in this important field. On the evidence collected and largely contributed by these two writers one may now state with confidence that albuminuria is nearly constantly present in pernicious æstivo-autumnal infections and is frequently observed in the milder tertian cases. Of greater interest and importance are the rather numerous records of distinct signs of an acute exudative process in the kidneys, usually in æstivo-autumnal cases, less often in tertian infections, and consisting in the presence in the urine of epithelial and granular casts, many blood cells, as well as considerable albumin. Although the milder changes in the urine may develop, according to Rempicci, either during or shortly after the acute attack, it would seem that the more severe urinary signs can be safely attributed to the malarial infection only when arising in the course of a pronounced acute seizure.

Recovery has usually followed the acute symptoms, but Kelsch and Kiener, Laveran, Marchiafava and Bignami, Rempicci, and Thayer have described various types of chronic nephritis occurring in long-established or chronic cases of malaria, while Kelsch and Kiener state that the chronic nephritis of malaria is distinguished from other types by the prominence of hemorrhages and the comparative absence of fatty and hyaline degeneration and of arterial changes.

The acute nephritis of malaria is almost never immediately fatal, so that the pathologist must speak with reserve regarding the origin and significance of many of the cases of exudative nephritis commonly attributed to malaria. In view of the frequent clinical reports of the presence of considerable albumin, many casts, and of blood cells in the urine, and less often of œdema and mild uræmic signs in the disease, it

[1] Read before the Association of American Physicians, Washington, May 2, 1901

seems certain that during severe malarial infection a true exudative nephritis is sometimes excited. Even in the absence of pathological examination of the kidneys these symptoms may with some confidence be referred to congestion of vessels, acute degeneration of cells, and exudation of serum and diapedesis of red cells from the bloodvessels, all dependent upon the action of the malarial toxin upon the renal tissue.

Coming, now, to the fatal cases of pernicious malaria in which there has been a microscopical examination of the kidney, the conditions found in this organ in all except haemoglobinuric cases have been very uniform. The lesions observed have been those of a severe acute degeneration of the cortical tubule cells, with moderate exudation of albumin into the tubules and glomeruli.

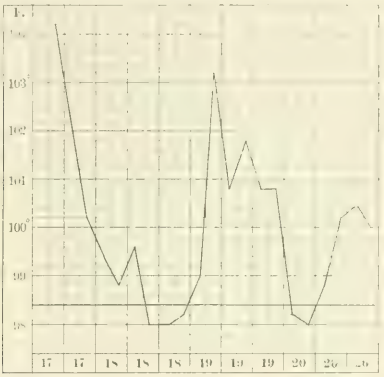
The chief contributions in this field have come from Guarnieri, Marchiafava and Bignami, Monti, Bastianelli, Barker, and Benevenuti. In all of the reports of these observers the nephritis has been clearly of toxic origin, while the number of parasites found in the kidney has been small, although they were usually very numerous in other tissues. The single exception to this rule is found in one of Barker's cases, in which death and probably the recent changes in the kidneys were principally referable to general infection by streptococcus pyogenes. Here the renal capillaries were frequently thrombosed by masses of streptococci and large numbers of tertian parasites. This case cannot, therefore, be regarded as one of malarial nephritis, but rather as an example of pyæmia or septicæmia terminating the usual benign course of a tertian malarial infection.

The invariable freedom of the kidney from localization of parasites in pernicious cases cannot be attributed to the phagocytic powers of this organ, which enjoys only a moderate capacity to destroy parasites. Guarnieri, who noted the small number of parasites in the kidneys of his and other cases, referred this comparative immunity to the rapid circulation of the organ.

The examination of the records of microscopical examinations of the kidneys in malarial fever fully warrants the latest conclusions of Marchiafava and Bignami regarding the origin of the renal changes in pernicious malaria, which are as follows, quoting from their article in the *Twentieth Century Practice:*

"As to the pathogenesis of the renal lesions in malarial infections, we are at present able only to form theories. The knowledge of the parasite has so far thrown no light on the pathogenesis of the nephritis. In pernicious infections very few parasites are found in the kidneys even when the changes in the epithelium may be so grave as to lead to necrosis. From this fact we may infer that the lesions are due not to a localization of parasites in the renal capillaries, but to some toxic substance eliminated by the kidneys."

My own experience in the examination of fourteen fatal cases of malaria, seen mostly at Montauk in 1898, fully accorded with the above conclusion, as I found, usually, extensive degenerative changes in the kidney, once the lesions of hæmoglobinuric malaria, but comparatively few or no parasites in the renal vessels. The present case, recently encountered, developing in the vicinity of New York, showing the clinical symptoms of acute hemorrhagic nephritis, with partial suppression of urine, and giving clear microscopical evidence of extreme massing of parasites in the kidney, with various mechanical lesions resulting from their presence, seems to require a reversal of the above conclusion, and appears to show that malarial nephritis may be caused principally or in large part by the presence of an excessive number of parasites in the renal capillaries.



Clinical History. The patient was a friendless girl, aged seventeen years, a Slav, who had been in America one year. She had enjoyed good health until going as a servant to College Point, Long Island, in July, 1900. She was discharged early in September on account of an alleged "inability to work," and this fact, together with a distinct pallor and slight jaundice, noted on her return to the city, indicate that she had suffered for some weeks from malaria contracted on Long Island. On September 12th she was taken acutely ill, with chills and fever, and, being without the help of a physician, she became steadily worse until brought by an ambulance to the hospital, on September 17th.

On admission the patient was well nourished; tongue moist, slightly coated, prostration marked. Physical examination of lungs, heart, and liver was negative. The area of splenic dulness was enlarged, but the organ was not palpable. There was slight œdema of the legs. The urine, voided naturally in moderate quantity, was described as of high color, clear, acid in reaction; specific gravity, 1018; urea, two and one-half grains to the ounce; albumin, 2 per cent. by volume; sugar

absent. Microscopically there were noted: epithelial cells, many red blood cells, and amorphous urates; diazo reaction marked. Temperature, 104.2°; pulse, 98. On September 18th there was vomiting of food and medicines; constipation relieved by enemas; and retention of urine with marked diminution in its quantity, which continued until death. Delirium developed on September 19th, and was controlled by morphine, sulphonal, and chloralamid, given at intervals, on the 19th and 20th. On the 19th she vomited a round worm and was given at intervals three doses of santonin, three, five, and fifteen grains, without any change being noted in the urine, stools, or conjunctiva. Delirium continued on the 20th, and, the pulse failing steadily, she became comatose and died at 9 P.M. The four-hourly temperature-chart is appended. The clinical diagnosis was typhoid fever, with acute nephritis.

Autopsy, by Dr. Otto Schultze, thirteen hours after death. Body well nourished; rigor well marked. *Heart* normal. An ounce of straw-colored fluid in pericardium. A few ecchymoses in visceral pericardium. *Lungs*: Few old pleural adhesions. A few ecchymoses in visceral pleura. *Liver* appeared normal. *Pancreas* normal. *Stomach* normal. *Ileum* congested, solitary follicles moderately swollen. *Colon* congested. *Spleen* much enlarged. Weight, sixteen ounces; consistence firm; Malpighian bodies prominent; pulp dark red. *Kidneys* together weighed sixteen ounces; consistence reduced; capsule free; cortex remarkably light in color; medulla and papillæ extremely dark, and slightly rust-colored; cortical markings entirely obscured. In the cortex of the right kidney there is a superficial anæmic infarct with surrounding hemorrhagic zone. This infarct measures $3\frac{1}{2} \times 2$ cm. in area, and 2 mm. in depth.

Anatomical Diagnosis. Acute hemorrhagic nephritis. There was still no suspicion of malarial infection. Cultures of liver, spleen, and kidney yielded bacillus coli and putrefactive species.

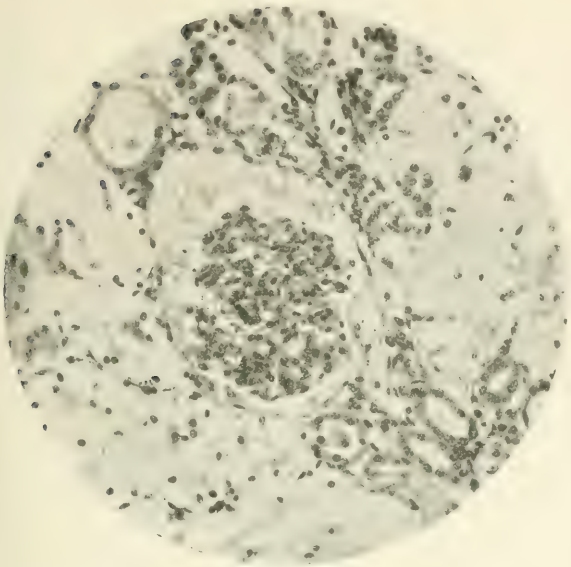
Through a misunderstanding, all the viscera, except the kidneys, were thrown away. The kidneys were hardened in 5 per cent. formalin and examined microscopically.

Microscopical Examination of Kidneys. After the malarial infection was suspected, on account of the peculiar pigmentation of the glomeruli and straight vessels, thin sections cut in celloidin and paraffin were stained most successfully by Nocht's method, as employed for blood smears. This was the only method by which I could secure a blue color in the bodies of parasites lying in red cells stained deeply by eosin. For this purpose sections remained in the dye for twenty-four hours, and were then slightly decolorized in alcohol, and cleared in oil of cajuput. The chromatin, however, was not stained. The changes demonstrated by this and other methods were of a most remarkable type.

The lining cells of the convoluted tubules were almost entirely destroyed, not by erosion, but by extreme swelling and degeneration, apparently of hydropic type. (Fig. 1.)

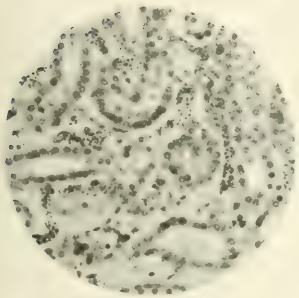
These tubules were thereby distended and uniformly filled by an indiscriminate mass of vacuolated cell detritus in which pyknotic nuclei were irregularly scattered. All the cortical tubules were about uniformly affected. The glomeruli contained a moderate number of pigmented cells and a few parasites within the capillaries, while the capsules were distended by granular coagulum. The small cortical vessels were almost invariably collapsed, and to this perfectly bloodless condition must be

FIG. 1.



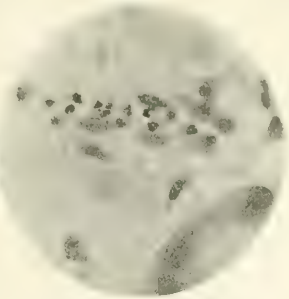
Convoluted tubules and Malpighian body. × 250.

FIG. 2.



Malarial parasites in kidney. 250

FIG. 3.



Parasites in renal capillary. 1000.

ascribed the whitish color visible in the gross. Occasionally a capillary was found distended with a mass of pigmented parasites.

In the medulla the lining cells of all tubules were markedly eroded. In this region most of the capillaries were filled, and many were distended with masses of infected red cells and pigmented parasites which had nearly destroyed the red cells. Fig. 2.) Many of the distended capillaries were ruptured, and the tissue was studded with miliary hemorrhages, about some of which a few leucocytes, mostly mononuclear, were gathered. The parasites were of small and very uniform size, but richly pigmented. They were identical in appearance with the full-grown forms of the æstivo-autumnal parasites seen in sections of tissue of other cases in which these parasites were identified in the blood during life. Very few rings or rosettes could be discovered, so that the brood was unusually compact. Fig. 3.) Their numbers and compact massing were similar to what I have seen in other fatal cases in which the parasites were massed in the brain, heart muscle, or bone-marrow. The discharging tubules were distended with casts, usually coarsely granular, sometimes entangling infected red cells and pigmented leucocytes. Some infected red cells lay free in the lowest discharging tubules.

The infarcted area in the right kidney was in an early stage of coagulation necrosis. All the capillaries about this area and many within it were distended with blood cells, most of which contained parasites and pigment. Many of the vessels leading to this area were distended to two or three times their natural calibre by thrombi of infected red cells. The infarction is clearly referable to the plugging of small vessels, mostly capillaries, by thrombi of infected red cells. The number of parasites in this cortical zone and throughout this section of the medulla was greater than I have ever seen in any other tissue.

The following *summary* presents the lesions observed in the kidneys: Extreme and peculiar degeneration of lining cells of all cortical tubules. Distention of capsules of glomeruli by granular albuminous coagulum. Occlusion of most cortical capillaries by the distended tubules. Large superficial red and white infarction, from occlusion of vessels by thrombi of infected red cells. Numerous miliary hemorrhages, from rupture of distended capillaries. Distention of nearly all capillaries in medulla and papillæ by infected red cells and parasites. Casts in discharging tubules, consisting usually of coarsely granular material, but sometimes entangling infected red cells and pigmented leucocytes.

Epicritical. The following questions arise concerning the significance of the above findings:

1. Is it possible to draw any conclusions regarding the significance of the renal lesions on account of the failure to examine the other viscera microscopically?

2. Is the present case a true example of massing of æstivo-autumnal parasites in the kidney comparable to the cases in which they have been found massed in the brain in comatose cases, in the gastro-intestinal tract in choleraic cases, in the heart muscle in cardiac cases, or in the marrow as observed in a case of pernicious anæmia?

3. What is the origin of the extreme degeneration of the cortical tubules?

1. To the first question there can be no hesitation in answering that the renal lesions are in themselves sufficiently distinct to stand as the chief feature of the case. Doubtless there were many parasites in the other viscera, but where pigmented parasites are extensively massed in any tissue gross evidence of their presence may be detected in the excessive pigmentation and extreme blood content of that tissue. No such evidence being present in the gross appearance of any other viscus, it is safe to conclude not only that the kidney contained an excessive proportion of parasites, but that they were not excessively numerous in any other locality. The clinical features of the case also support this belief.

2. It is equally clear that the case is one of true malarial nephritis in which a peculiar hemorrhagic character of the lesion is referable to the mechanical effects of massing of parasites in the renal tissue. This lesion is not to be classed with the usual type of renal lesion in malaria, which is an acute degeneration referable to the general toxæmia of the disease, and in which comparatively few parasites have heretofore been found in the kidney. Enormous numbers of parasites in the capillaries, thrombosis of many vessels, and multiple hemorrhages are the specific lesions observed when the parasites have been massed in the brain, gastro-intestinal tract, heart muscle, and marrow.

3. The extreme degeneration of the cortical tubules seems also to be a somewhat peculiar feature of the present case. In the reported cases of malarial nephritis of purely toxic origin the degeneration of tubule cells has often been extreme, but it has always been of a different type from that described above. In fourteen cases which I have examined the degenerative changes have always been much less advanced and of a different type from those of the present case. Although much of the change in the tubule cells was doubtless owing to the general toxæmia of the disease, I am inclined to refer the peculiar character of the process and its extreme degree to the presence of so many parasites in the tissues acting principally through obstruction of the circulation.

Finally, it is important to consider the possibilities of diagnosis during life of this type of malarial nephritis. From the clinical examination of the urine and the microscopical appearance of the discharging tubules in the kidney it seems probable that a diagnosis of this type of malarial nephritis may be established during life on the following evidence, to be obtained from the urine. Partial or complete suppression of urine; considerable admixture of intact red cells; a considerable proportion of albumin; the presence of many coarsely granular, epithelial, and some blood casts; the presence of *infected red cells*, both free and adherent to casts, and of pigmented leucocytes adherent to casts.

The present case, while demonstrating the existence of a specific type of malarial nephritis, yields no new indication why the æstivo-autumnal

parasite selects certain viscera for its chief attack, and demonstrates no new character in the pathogenic action of this protozoon.

There is no known reason why the bronchopulmonary mucosa, or that of the colon, or the serous membranes, or the skeletal muscles, should not suffer in the same way as do the brain, the gastro-intestinal mucosa, the heart muscle, and the marrow. The kidney may be specially exempt on account of its rapid circulation, as suggested by Guarnieri, but apparently it does not always escape. The actively phagocytic viscera, the liver, and spleen, seem capable of rapidly destroying the enormous influx of parasites which frequently reaches their capillary systems. Yet the lymphoid marrow, with its more complex circulation, is known to be less resistant than the spleen and lymph nodes.

These two factors, however—rapid arterial circulation and active phagocytic powers—seem to be the only qualities which can preserve a tissue from occasional overwhelming growth of the æstivo-autumnal parasite.

Microscopical examination of the kidneys of fatal cases of malaria has, then, yielded evidence of three main types of acute renal lesions occurring in this disease.

1. Acute degeneration of toxic origin, often reaching a degree in which exudation of blood serum into the tubules is added. This lesion is responsible for the vast majority of the cases of albuminuria in malaria.

2. An extreme form of acute degeneration, with focal necroses, numerous hemorrhages, and exudation into the tubules of blood serum and blood pigments. This lesion is seen in cases of hæmoglobinuric malarial fever, and it has not yet been found associated with an excessive number of parasites in the capillary vessels.

3. Massing of parasites in the renal capillaries, with extreme degeneration of parenchyma cells, multiple hemorrhages, and exudation of blood serum into the tubules. It seems certain that this type of lesion can occur only in severe æstivo-autumnal infections.

There is anatomical evidence that in the pernicious æstivo-autumnal cases the three types of lesions may be variously combined, but no good reason for believing that with the benign tertian infection occurring in this latitude any other than the first type can exist.

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GIANT CELLS.[1]

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UNDER ordinary conditions cells divide by the well-known mitotic processes, and this applies to those of the lower organisms as well as of the highest: the notion, previously held, that amitotic represents the primitive method, and mitotic a later improvement on this, being now abandoned. Amitotic division is comparatively uncommon, and its occurrence is, by most authorities, held to be an indication of approach to degeneration: the resulting tissue being short-lived, although for a time the increase in numbers of the cells may be extremely rapid.

Physiologically amitotic cell-division has been observed to occur in the ovarian follicle cells of certain insects and crustaceans by Korschelt and Carnoy, whilst vom Rath (1893) has noticed it in the supporting cells of the testis, but never in the spermatogonia of vertebrates, mollusks, and anthropods. Meves (1894) differs from vom Rath and claims to have found amitoses in the spermatogonia of the salamander: this, however, occurring but seldom, and chiefly in the colder months, so that it seems quite probable that the resulting cells are not active sparmatozoa.

[1]Photographs taken by B. H. Buxton, M.D., at the Loomis Laboratory, Cornell Medical College, New York, from original specimens prepared at the General Memorial Hospital. A Powell and Lealand ¼-inch oil immersion was used for the magnifications of 250 diameters, and for that of 700 diameters a Spencer $^1/_{10}$ inch brom-naphthalin immersion. Reproduced in Alberttype by the Alberttype Co. of Brooklyn, N. Y.

In these instances the cells are transient in nature, and only for a comparatively short time perform their special functions.

Pathological.—Leucocytes increase normally by mitosis, but in inflammatory processes, where they are suddenly required in immense numbers for a temporary purpose, they increase amitotically. Their functions under these circumstances appear to be to break down intercellular substances, neutralize toxines and other poisons, by means of their secretions, and finally to carry off débris by means of their phagocytic properties. Having done this and cleared the way for the regenerative cells, they die and are absorbed.

It appears that cells may occasionally divide amitotically for a time and then resume mitosis. Preusse (1895) considers this to be the case in the cells of certain insect ovaries, whilst Pfeffer (1890) shows that "if spirogyra be placed in water containing ½ to 1 per cent. of ether, active growth and cell division occur, but only amitotically. If, however, the plant be replaced in pure water, mitotic division is resumed, and normal growth continues." Wilson (1900) from whom this is quoted, concludes that amitosis does not necessarily mean the approach of degeneration, at any rate in the lower forms of life, but only a special condition, though a fairer inference would seem to be that the tendency to degeneration has not proceeded so far that it cannot be recovered from on a return to normal conditions. The experiment seems to confirm the degeneration hypothesis rather than to render it doubtful.

Some steps nearer to the approach of degeneration are those cases where amitotic nuclear division is not followed by cell-division; the resulting tissue consisting of a continuous protoplasmic mass or syncytium, containing numerous nuclei.

Physiologically, we find such a tissue in the syncytium of the embryonic chorion villi. It is not yet determined if this syncytium is of maternal or fetal origin, Kossmann and Pels-Leusden (1897) being the chief upholders of the former view, whilst Marchand (1898), Aschoff (1898), and Minot incline toward the latter. Whatever its origin, however, it is beyond dispute that the syncytium of the chorion forms the line of demarcation between mother and fetus, so that all the nourishment and oxygen received by the latter has to pass through the syncytial layer. It is, therefore, essential that this increase very rapidly, so as to quickly afford an extended surface, and since it is only required for a short period of time it is immaterial if it contain the elements of early decay. The nuclei therefore divide amitotically with great rapidity, and the whole structure is in a condition of degeneration at the time of parturition. It is doubtful if any part of the syncytium actually lives during the whole nine months of gestation, since it seems,

according to Minot, to be in a constant state of decay and renewal. It is not improbable that the syncytium is also of use in providing a continuous protoplasmic surface for absorption instead of one with a series of breaks and consequent possible leakage.

Pathological.—In a very interesting article by Loeb (1897) on regeneration of the epidermis, he shows that this is effected in what may be termed a duplicate fashion. In the first place the upper layers of the rete Malpighii around the margins of the denuded surface began to proliferate. Their nuclei divide amiototically and this is not followed at once by cell division, though it occurs imperfectly later on. At first a syncytium is formed which rapidly covers the surface of the wound, but this is only a temporary covering, being gradually replaced from below by cells derived from the basal layer, which divide in the ordinary way by mitosis, and by means of which a fresh permanent layer of epidermis is formed. Here again we have amitotic nuclear division and formation of a syncytium where rapid growth is essential without, however, any necessity for permanence. Loeb's views are certainly not generally accepted, but he appears to have made a more careful study of the process than most observers.

Coming now to the subject-matter of the article—giant cells—we find that in their early stages, whilst the nuclei are still few in number these may divide mitotically (Goldman, Baumgarten, Manasse), though the mitoses are apt to be multipolar and otherwise irregular. Other observers (Marchand, Von Bungner, Welcker) could find none even in early stages. All agree, however, that later on, when the nuclei become more numerous, and the giant cell, as such, is formed, division takes place amitotically only. This nuclear division not being followed by cell division it is evident that multinuclear giant cells must be looked upon as essentially syncytial in nature, and as containing in themselves the elements of early decay. It is true that giant cells do not show the rapid growth so characteristic of the syncytia already mentioned, but Henneguy (1896), following Herbert Spencer, clearly shows that where cells retain their original spherical or polyhedral shape, with increase in bulk, the ratio of surface to cell contents decreases by geometrical progression, and that such cells become less active and capable of division as they grow larger. He compares bacteria with amebæ.

In the syncytia of the chorion and regeneration of epidermis the lateral growth enables the usual proportion between surface and cell contents to be maintained, so that rapid increase is possible, whilst giant cells being, no doubt, prevented by the surrounding tissue from spreading laterally, retain practically their original shape, so are slow

of growth and limited in size. The prolongations they often exhibit may perhaps be taken as representing attempts to attain an increase of surface.

Although some observers (von Rindfleisch, 1894) have maintained that giant cells occurring in chronic inflammatory processes, etc., may become organized and help to form scar tissue, yet the balance of opinion is adverse to any such views, and it is generally held that a giant cell, whether physiological or pathological, is a giant cell, that it lives for a time as such, and as such it dies.

It appears, however, that in an early stage the growth of giant cells may become arrested: they may break up into cells resembling the parent cells, and resume their normal career. Once, however, the nuclei have begun to divide amitotically there is no return possible. There seems to be some analogy between this, and the results obtained in the spirogyra experiments.

The disposition of the nuclei in giant cells varies, and may be considered to fall into two groups, between which, however, no hard and fast line can be drawn.

1. *Myeloid Type.*—The nuclei are grouped centrally or irregularly distributed. Typical in cases where inert, indigestible material is present. May be physiological or pathological.

2. *Langhans Type.*—The nuclei are grouped at the periphery of the cell: either all round the margin, or at one of the poles. Typical in chronic inflammatory processes. Always pathological.

On consultation of the literature pertaining to the subject of giant cells especially so far as it relates to those found in tuberculosis, which may be taken as typical of all slowly progressive mycotic inflammations in which giant cells are formed, we find opinions greatly divided: the following table representing the extreme views on either side, which generally speaking, may be called those of the French school and of the German school.

Progress of Tuberculous Infection in Regard to the Formation of Giant Cells.

French School.	German School.
Metchnikoff, Borrel, etc.	Weigert, Marchand, etc.
1. Invasion of small mono- and polynuclear leucocytes, which are:	
phagocytic.	not phagocytic.
2. Advent of larger mononuclear formative cells:	
Of leucocytic origin.	of connective tissue or endothelial origin.

3. Which form giant cells: by grouping themselves around masses of bacilli, and fusing. No nuclear division.	by amitotic nuclear division of a single cell.
4. The giant cells have ameboid movements.	no ameboid movements.
5. They are phagocytic.	not phagocytic.
6. The protoplasm of those parts of the cell destitute of nuclei is specially active, whilst the nuclei are grouped in the less active parts.	has undergone partial necrosis, whilst the nuclei are grouped in those parts of the cell which have retained their vitality.

This table represents what may be called the views of the extremists on either side, but between these are to be found every possible modification: some others inclining to one side and some to the other, whilst there is no absolute and entire agreement between any two of them.

At this point one is forcibly reminded of Mark Twain's sensational novel, in which he got his hero into such a fearful tangle of complications that he had to leave it to his readers to get him out of it as best they could. But it will be more interesting to face the music and critically examine the situation from the various points of view.

Before taking up the points mentioned in the table it may be well to consider the formation of physiological giant cells as found in bone, and of the so-called foreign body giant cells.

Bone, especially in the earlier stages of its formation, is continually passing through periods of resorption and reconstruction, with formation of Howship's lacunæ, in which giant cells (myeloplaxes), clinging to the surface of the bone where resorption is proceeding, are constantly found, and outside of these a zone of granulation tissue. The earlier observers held that the myeloplaxes were the chief agents in the resorption processes and Kolliker named them osteoclasts. This position has been much contested of late, and it seems more probable that the resorption is due to the activity of the granulation tissue, of which the myeloplaxes are modified portions. The granulation cells lying nearest to the hard, inert surface of the bone, and being pressed up against it by the invading tissue, are not bathed in lymph from all sides as they would naturally be. Nutriment therefore is insufficient, and this leads to imperfect cell division. The giant cells, no doubt, are active agents in so far as they are a part of the granulation tissue, but are not in themselves the specific cause of the resorption.

It is true that the myeloplaxes are found throughout life in the bone marrow, but they usually show signs of fatty degeneration, and do not give the impression of durability. It is probable that they are being constantly formed wherever resorption is proceeding, and after formation float off into the marrow, where they exist for a time, and gradually disappear. Meyer (1803) takes this view of their origin, although he believes in their specific functions. The opposite view that the giant cells of the marrow are a reserve fund of osteoclasts, which multiply as occasion arises for bone resorption, may be correct, but cannot be held by any one who believes that the nuclei of giant cells divide amitotically, and that the cells themselves are of a syncytial nature, since this belief would exclude the possibility of their durability and power of originating similar independent cells.

In pathological lacunar absorption the processes are similar, but it is significant that in occasional instances where absorption is proceeding very rapidly, there is formation of Howship's lacunæ filled with granulation tissue without giant cells (von Recklinghausen, 1891). One can imagine that where pressure on the cells lying next to the bone is rapidly relieved there is no interference with nourishment and, therefore, no imperfect cell division.

Foreign Body Giant Cells.—Where some extraneous material is inserted in the tissues, either accidentally, surgically, or by way of experiment, there is at first leucocytic invasion, quickly followed, if there is no suppuration due to bacterial infection, by the appearance of formative granulation tissue, which soon surrounds the substance. If this latter be soft and easily absorbable, as lung or liver tissue, there are no giant cells formed, but if it be hard or indigestible, such as a piece of sponge, catgut, or bone, with the advent of the granulation tissue, giant cells make their appearance, closely hugging the surface of the foreign body.

The reason for their appearance seems to be the same as in the case of bone absorption: the granulation cells nearest to the foreign body, being pressed closely against it by the actively increasing tissue, are insufficiently nourished with resulting abnormal nuclear and incomplete cell division.

That leucocytes of the early invasion give rise to the giant cells in foreign body intrusion seems unlikely for:

1. Giant cells do not appear until the advent of granulation tissue.

2. Their formation begins at the periphery of the foreign body in the granulation zone.

3. Their nuclei and cytoplasm resembles those of the granulation cells.

4. Some leucocytes penetrate the foreign body and come to a standstill there. Here would be the place for them to form giant cells if they could, but these are found at the periphery only, and never inside the foreign body (Hamerl, Von Bungner).

Krückman and others maintain that epithelium may occasionally give rise to giant cells, and there seems no reason to suppose that their conclusions are incorrect.

Returning now to the table showing the extreme views on either side as regards the processes of giant-cell formation in tuberculosis, each point can be taken up separately.

1. Invasion of small mono- and polynuclear leucocytes.

All agree that this is the first step in the attempted destruction of the tubercle bacilli, and the question of their phagocytic properties need not concern us, as no one, except perhaps Arnold, supposes that they give rise to giant cells.

2. Advent of large mononuclear formative cells.

Since it is agreed that it is from these that the giant cells are formed, the question of their origin is of interest. Metschnikoff; Borrel, and Arnold claim that they are of leucocytic origin.

Borrel (1803 and 1894) in his experiments injected tubercle bacilli into the ear veins of rabbits, and found the bacilli were intercepted in the capillaries of the lungs, where tubercles were quickly formed; at first in the capillaries and later in the alveoli. He observed that the larger mononuclear cells appeared in the capillaries in forty-eight hours, and argued from this that they must be of intravascular origin and, consequently, leucocytic. Cornil and Yersin have arrived at the same conclusions. Arnold (1893), studying inflammation in the mesentery of frogs, noticed the appearance in twenty-four hours of mononuclear spindle and branching cells, and concluded that in such a short space of time these could not have been formed from the fixed cells, but must necessarily have arisen from the leucocytes of the early invasion. Arnold concedes, however, that the granulation tissue of leucocytic origin is probably only temporary, being replaced later by tissue derived from the fixed cells. Formerly Ziegler and many German observers held similar views, but have now abandoned them in favor of the origin of the granulation from the fixed cells of the connective tissue in which are included the endothelium of the vessels, lymph spaces and serous cavities. They admit, however, that the connective tissue cells of young granulation tissue possesses mobility and that at this stage it is difficult to judge with certainty between them and the large mononuclear lymphocytes, although there are differences in the appearance of the

nuclei, the cell protoplasm, and mitoses by which they can be distinguished.

When we speak of round-cell infiltration it must be confessed that under this appellation both leucocytes and early granulation cells are included, between which no differentiation can be made by the ordinary observer. As Unna justly remarks: "The expression round-cell infiltration is a cloak made to cover many gaps in our knowledge."

It does not seem inherently impossible that leucocytes should be able to give rise to connective tissue. It must be remembered that in invertebrates, leucocytes and mesodermal cells are one and the same thing, whilst in vertebrates they are both derived from the ameboid mesenchymal cells of the early embryo. On further development those mesenchymal cells, which remain in fluid media, such as lymph and blood, continue their ameboid existence, whilst others which find themselves in the gradually thickening mesenchymal substances become fixed connective-tissue cells. In inflammatory processes the intercellular substances are broken down by the action of the leucocytes, and in consequence of this the connective-tissue cells are set free in a fluid medium resembling that of the embryonic mesenchymal fluid. They now take on ameboid movements and, mingling with the leucocytes, cannot be distinguished, at any rate from the large mononuclear ones, although, as already mentioned, some German authors claim that there are certain differences between them. In any event it seems that connective-tissue cells do not become very highly differentiated, but can readily adapt themselves to their environment: returning, as it were, on occasion to their early embryonic forms. Per contra it is not unreasonable to suppose that leucocytes, whose origin is the same as that of the connective-tissue cells, may also be capable of adaptability, and, given a suitable environment, can take upon themselves the characters and functions of the latter. It is certainly improbable, though not inconceivable, that leucocytes and connective-tissue cells, are interchangeable.[2]

3. Origin of giant cells.

The extreme view held by Borrel that they are formed by fusion of several cells without any subsequent division of nuclei does not seem to be shared by any one else, and it may be taken for granted that there is, at any rate, amitotic division of the nuclei after the commencement of formation of the giant cell. But the question as to whether they arise from one cell or by fusion of several must be considered as still "*sub judice*." The conclusions of some authors may be given, but it

[2] Since writing this Beard (Anatomischer Anzeiger, 1900) and others claim to have traced the origin of leucocytes to the epithelium of the thymus gland. If this view proves correct, the above arguments fall to the ground.

hardly seems worth while to recapitulate their arguments in favor of their views. For the most part they are pious opinions and nothing more.

One Cell.—Goldman, Welcker, Birch Hirschfeld, Baumgarten.

Several Cells.—Von Bungner, Borrel.

One Cell, or several, according to circumstances.—Manasse, Metschnikoff, Krückmann, Ribbert.

The older pre-tubercle bacillus view of Ranvier and Cornil (1878) that giant cells arise from metamorphosed vessels—the giant cell with the surrounding epithelioid cells representing an obliterated capillary—is now abandoned, though Borst (1896) seem to think it may be true in some instances.

4. Ameboid properties of giant cells.

Metschnikoff did not attempt to observe giant cells in the living condition on the warm stage, but the long protoplasmic processes which they exhibit in fixed preparations he considered as pseudopods, and concluded that the cells possess mobility.

Weigert (1888) Welcker (1895), and others who have observed giant cells on the warm stage, have been unable to detect any such ameboid movements as are shown, for instance, by leucocytes, and from the appearance in fixed preparations of what are known to be ameboid cells we can only conclude that true pseudopods cannot be fixed. Leucocytes appear as spherical cells, the pseudopods evidently being retracted at the moment of fixation; *i.e.*, death of the cell.

An illustration may be taken from the early embryo. After the establishment of the three primary germ layers, all of which are epithelial in character, there is serous effusion between the middle and two outer ones. Cells now detach themselves from the middle layer or mesoderm, and, taking on ameboid movements, wander into the effusion or mesenchymal fluid: being now no longer epithelial but mesenchymal cells, from which, for the most part, connective tissue will be formed. At this stage such ameboid cells in fixed preparations appear spherical. Later on, the mesenchymal fluid becomes thickened and mucilaginous, so that the cells can no longer wander, but become fixed, their pseudopods forming elongated protoplasmic processes which anastomose with those of other similar cells and cannot now be retracted. In fixed preparations these processes are visible, affording with the cell bodies and intercellular substances, the well-known appearance of mucoid tissue.

The long processes of the giant cells, therefore, are almost certainly fixed, being probably continuous with the reticulum of the tubercle, and cannot be retracted. Schmaus and Usinsky (1894)

claim to have demonstrated that many of the prolongations of the epithelioid and giant cells in tubercle anastomose, and contribute to the formation of the intercellular network or reticulum. The presence of such prolongations in fixed preparations is an argument against the mobility of giant cells, and we may fairly conclude that these have no ameboid movements.

5. Phagocytic properties.

If it be conceded that giant cells are destitute of mobility, phagocytic properties must also necessarily be denied to them.

6. Disposition of nuclei, etc.

This question may be said to resolve itself into a battle royal between Weigert and Metschnikoff, other writers on the subject contenting themselves with applause or dissent. Weigert in 1885 opened fire by attempting to account for the peripheral arrangement of the nuclei in cells of the Langhans type found in tuberculosis, by supposing that, in consequence of the action of the toxines, elaborated by the bacilli, the center of the cell has undergone partial necrosis. The nuclei consequently group themselves at the periphery or at one pole, where the cytoplasm is still active. He points out that in comparatively early stages, whilst the bacilli are few in number, they are found in the center of the cells with the nuclei at the periphery (commencement of necrosis).

Later on, the bacilli become more numerous and take on a radial arrangement, just internal to the nuclei and pushing in between them, whilst none are found in the center.

This he accounts for by supposing that owing to progressive necrosis at the center, nourishment becomes exhausted there, and the bacilli work their way towards the periphery.

The center of the cell at this stage stains but faintly, and this he also takes as evidence of degeneration.

Metschnikoff, in defense of his favorite phagocytic theory, points out that in the giant cells some of the bacilli take the red or violet stain very poorly, whilst in the center of the cell are small, yellow masses which he supposes to be the remains of dead bacilli or rather of sheaths secreted by them before death as a means of defense against attack by the cell.

Koch had already observed these yellow bodies and, regarding them as some of the original infecting bacilli which had died a natural death of old age, argued in favor of a certain durability of the giant cell.

Metschnikoff held that they represent bacilli which have been killed by the phagocytic properties of the cell, and maintained that the center is the more active part, whilst Weigert, in reply, contended that they

are probably not remains of bacilli at all but altered portions of the cell protoplasm.

There seems little doubt, however, that Koch and Metschnikoff were correct in their observations, and Weigert's safest retort would have been that the yellow bodies are bacilli which have been killed by the action of their own toxic products.

Metschnikoff does not seem to have dwelt much upon the faintly staining qualities of the cell center, but his follower, Borrel, maintains that this is due to the large quantities of digestive material present, and instances the case of goblet cells as analogous. This certainly is rather far-fetched, and Weigert seems to have the best of the argument: the necrosis theory much more readily accounting for the actual appearances than the opposite view. The question may be also approached from what might be called the phylogenetic standpoint.

In physiological giant cells we find the nuclei always grouped in the center of the cell—the typical myeloid cell. The cytoplasm being equally active throughout, the nuclei take up their natural position in the center.

In inert foreign body intrusion giant cells are formed, some of which have the typical myeloid appearance. In others the nuclei are irregularly scattered throughout the protoplasm, whilst others again approach the Langhans type, though it is reserved for tuberculosis and other infective granulomas to show this type to perfection. We can readily suppose that bone being a part of the organism, there is nothing set free from it on absorption, which is actively injurious to the cells: the giant cells being formed simply on account of lack of proper nourishment.

An extraneous foreign body, on the other hand, is likely to contain substances which, on being set free by gradual absorption, would act deleteriously on the cells. The giant cells are formed in the first instance from lack of nourishment, and later suffer more or less from the action of such substances upon them. The greater the injury to the cells the greater the likelihood of some portions becoming necrosed. From such portions the nuclei would recede, and the cell approach the Langhans type.

In tuberculosis and similar infections it is probable that where one or a few micro-organisms come to a standstill, the first granulation cells which appear find their nourishment sufficiently interfered with by the toxines to prevent their normal increase by division. A giant cell is then formed either by fusion of several or increase of a single cell; if the latter, probably the one into which the bacilli first penetrate. At first the giant cell does not necessarily assume the Langhans type, but

as the bacilli increase in number their toxic products cause progressive necrosis at the center and the peripheral arrangement of the nuclei becomes more marked. Generally speaking, therefore, the greater the injury to the cell the nearer does it conform to the Langhans type.

Giant Cells in Tumors.—There are certain sarcomas arising in connection with bone marrow, which contain multinuclear giant cells, resembling the normal myeloplaxes in every respect. Evidently such cells must be derived either from pre-existing myeloplaxes, or, if the possibility of these undergoing cell division be denied, from fixed connective-tissue cells, represented in the tumor by spindle or round cells, which are numerically always greatly in excess of the giant cells. The giant cells themselves, however, are often found in large numbers and the only way in which the latter hypothesis can be made to account for their presence is by supposing them to be formed at the margins of the advancing tumor where the bone is being resolved, and after formation remaining among the ordinary tumor cells whilst the bone recedes on account of the progressive resorption, the giant cells existing for a time, but probably taking no active part in the further development of the growth.

The giant cells in such tumors do, as a fact, very quickly degenerate, often showing vacuolation and alteration in the nuclei, before there is evidence of regressive changes in the surrounding spindle or round cells.

Tumors derived from the bone itself also frequently show myeloid giant cells, but in fewer numbers, whilst periosteal sarcomas almost invariably contain so-called giant cells, which have, however, nothing in common with the multinuclear giant cells under consideration in this article. They are large, spherical, or irregularly-shaped cells with one, or perhaps two or three, enormous nuclei, showing excessive nuclear hyperchromatism, which Hansemann (1897) ascribes to a process of karyorhexis. It is uncertain if this view be correct or not, but intense hyperchromatism is a not uncommon accompaniment of malignant tumors, whether sarcomas or carcinomas. Such periosteal sarcomas are usually exceedingly malignant, whereas the true myelosarcomas or those in which the giant cells resemble the myeloplaxes, can hardly be considered as exhibiting malignancy at all, being of slow growth, and seldom recurring or forming metastases.

A specially benign type is that known as epulis, proceeding from the alveolar process of the jaw.

Sarcomas other than those arising in connection with bone may

Periosteal sarcomas do not burrow into and absorb bone like the myelosarcomas, but grow outwards along the lines of least resistance, displacing and replacing connective tissue and muscles.

contain giant cells. Krückman (1895) describes two such cases: in one of which the giant cells were grouped around masses of blood-pigment and in the other around hyalin material. For these and certain other reasons he concludes that the giant cells are of foreign-body origin, playing merely a secondary rôle in the formation of the tumor.

Carcinomas also occasionally contain giant cells, especially the epitheliomas of the skin and stratified mucous membranes. In these cases the giant cells are often found surrounding horny epithelial pearls in which active growth appears to have ceased, so that these may be considered in the light of inert foreign bodies. Giant cells may sometimes also be observed scattered in the granulation tissue which so often accompanies carcinomatous infiltration, and in such instances, according to Krückman and Lubarsch, careful search will usually reveal fragments of dead epithelial cells, cholesterin crystals, etc., in close proximity to, or contained within, the cells, so that a foreign-body origin may be ascribed to the latter.

Manasse[11] (1894) describes nine cases of granulation polyps of the ear, in almost all of which giant cells were found, and decides from the appearances noted that these were of foreign-body origin, and had nothing to do with the formation of the polyps as such.

Without quoting other authors who reach similar conclusions we may sum up that giant cells:

1. In myelosarcomas represent absorption of bone.
2. In other tumors represent foreign-body intrusion.
3. In all tumors are a secondary manifestation, and do not actively contribute to their growth.

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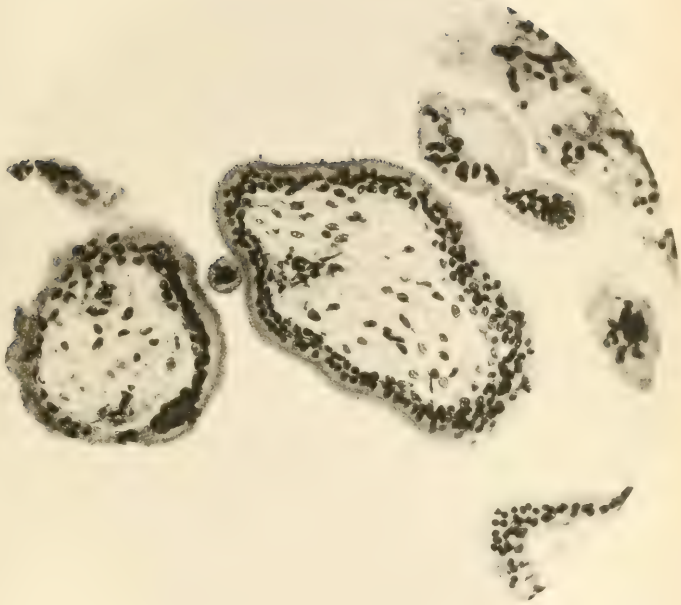

Chorionic villus from human ovum twenty-five days old, showing syncytium. X 250.

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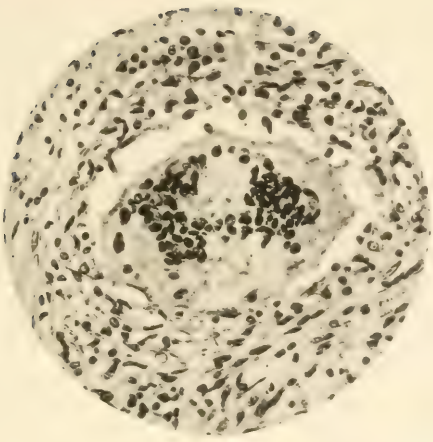

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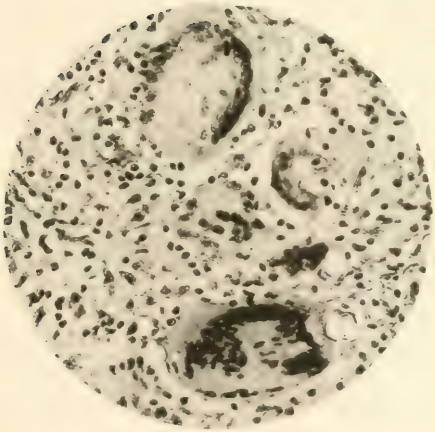

b.

a. Early stage of giant cell formation in miliary tubercle of pulmonary tuberculosis. As yet there is no peripheral arrangement of the nuclei. X 250.

b. Later stages of giant cells from a case of Lupus showing the Langhans type. X 250.

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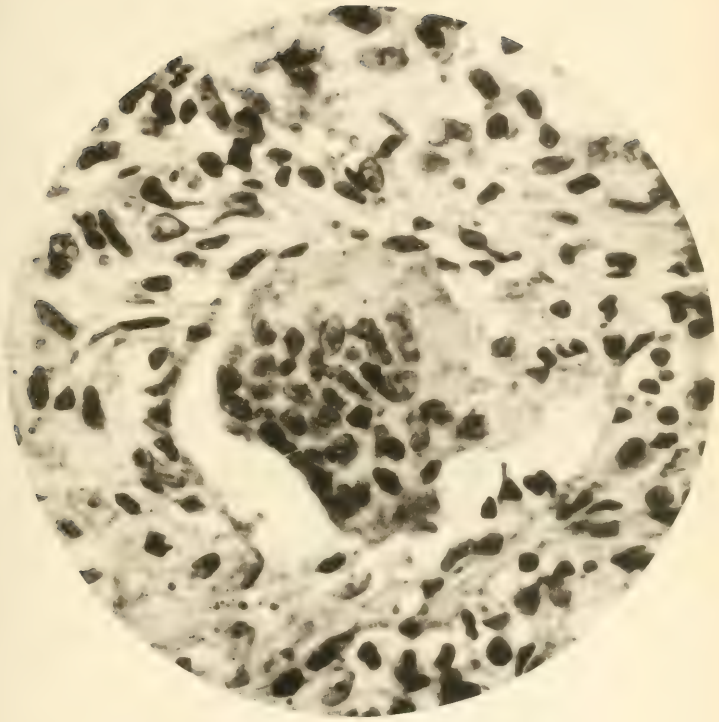

A myeloid giant cell from a case of Epulis. X 1000.

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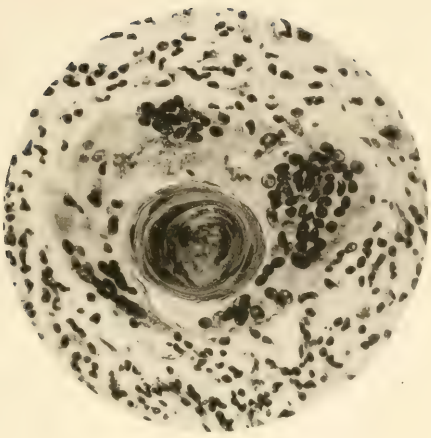

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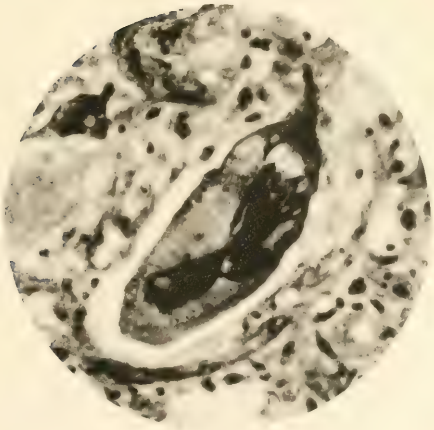

b.

a. Myeloid giant cell sarcoma, the [illegible]

b. Large—miscalled giant—cell from a periosteal sarcoma of thigh showing intense nuclear hyperchromatism. X 250.

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BENIGN EPITHELIAL TUMORS OF THE SKIN.[1]

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IT will be well to define at the outset what is the meaning of the word epithelial, and for this purpose the reader is referred to a previous article in the February, 1900, number of this Journal, where reasons are given for considering as epithelial those cells, no matter what their origin, which, lying closely packed together, without any intercellular substance beyond an exceedingly thin sheet of mucilaginous cement between them, constitute definite layers, no vessels penetrating between the individual cells.

Such layers line the external and internal surfaces of the body, the lumina of gland alveoli, ducts, and vessels, or they may be arranged in cords, as in the liver and suprarenals.

Under this definition it is evident that not only the cells of the epidermis and its derived glands, but also the endothelial cells lining the blood and lymph vessels, are to be considered as epithelium. These latter have, in fact, all the characteristics of epithelium, and tumors arising from them have the characteristics of epithelial, and not connective tissue, tumors, although, like the connective tissue cells, they

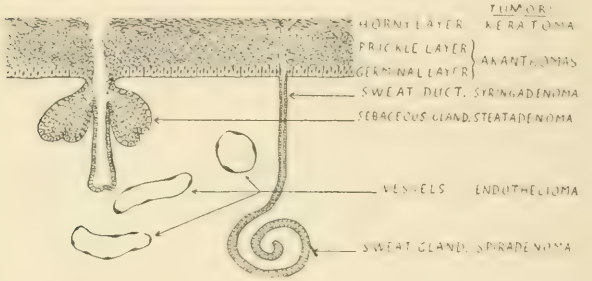
[1] Photographs by the author, partly from original specimens examined at the General Memorial Hospital and partly from those supplied by Drs. Schultze and Johnston.

Up to 10 diameters, Ross anastigmatic.
25 " Spencer, 2-inch photographic.
250 " Powell and Lealand. ¼-in., apochromatic oil immersion.

are derived from the mesenchymal cells of the early embryo, and not directly from any one of the three primary epithelial germ layers.

Tumors, therefore, arising from endothelium, situated in the corium, must be held to be of an epithelial nature, and as such will be dealt with in this article. On the other hand, pigmented and other nævi and tumors derived from them will be excluded. Such tumors have usually been regarded as of connective tissue origin, but Unna, with whom Gilchrist and many other recent writers seem to be in accord, claims to have traced their origin from epithelium of the ectoderm, which has been cut off from its parent stock in the process of development, and remains heterotopically imbedded in the connective tissue of the corium.

Lubarsch, who revels in contradiction, and who may be called the pathological Mephisto, "Der Geist der stets Verneint," was unable to



find any such snaring off of epithelial cells, and roundly asserts the connective tissue origin of cells found in nævi. Bauer (*Virchow's Archiv*, Vol. 142) and some others hold that nævus cells are endothelial, but as the question is still an open one, it may be provisionally assumed that they are modified connective tissue cells, probably allied to the chromatophores, since they so frequently contain pigment, and will, therefore, be excluded from consideration in this article. Nor will retention cysts or tumors arising in connection with the highly specialized mammary gland be dealt with.

In spite of these exclusions there is plenty of material left to work upon, as may be seen by a glance at the accompanying diagram, in which are represented the various layers of the epidermis and its derived glands, together with an enumeration of the tumors derived from them.

For cutaneous warts and similar growths, the term papilloma is in general use. This word was coined in the early days of pathology, and expresses the idea, then prevalent, that such growths are due primarily to increase in size and numbers of the papillæ. To-day, however, it is generally recognized that the papillæ, as such, play an altogether secondary rôle in the formation of true warts, and that these are due, in the first instance, to proliferation of the epithelium. The word papilloma, therefore, should be dropped in a discussion of the subject of cutaneous growths, although it may conveniently be retained for the purpose of reporting pathological examinations where the chief object is to express the idea of benignity, as opposed to malignancy, and should be used in a general way as descriptive of excrescences arising from a surface, whether primarily of epithelial or connective tissue origin.

Unna distinguishes two types of tumors arising from the epidermis: the keratomas, in which the horny layer is most conspicuous, and the akanthomas, which are chiefly made up of prickle cells, the horny layer being comparatively insignificant.

In the keratomas, under which are included corns and cutaneous horns, the increase of the tumor is, of course, due merely to apposition, there being no actual growth of the dead horny cells themselves. These, instead of peeling off, for some reason or other remain glued together, and beings constantly reinforced from below, a warty growth or cutaneous horn is gradually formed.

In the corns, constant pressure from without prevents the usual desquamation of the dead surface cells, which, consequently, form a hard, thick layer, followed secondarily by some increase of the prickle layers. The formation of the cutaneous horns, on the other hand, is probably due to some abnormality in the cells themselves: the horny layer and rête Malpighii increasing simultaneously, the former, however, being by far the more conspicuous, so that cutaneous horns can be distinguished micro- as well as macroscopically from ordinary warts.

Plate I.*b* shows a keratoma from a male patient aged 60. For ten years there had been a small, painful spot under the right eye, which had become ulcerated four weeks before removal.

A plug of horny material can be seen in the center, extending inward as well as outward, so that the growing prickle layer has been pushed down into the corium and beneath the normal epidermis on either side, but there is no indication of infiltration of the underlying tissues, such as would be observed in a malignant growth.

Of the akanthomas, or growths arising from the rête Malpighii,

four varieties may be distinguished: (1) Verruca vulgaris, (2) condyloma acuminatum, (3) molluscum contagiosum, (4) akanthoma adenoides cysticum.

1. *Verruca Vulgaris* (Plate I.*a*, wart on lip).—The horny layer is increased in thickness, though but little, by comparison with the rête Malpighii. The rête cells themselves are rapidly replenished from the germinal layer, where mitoses are frequent, whilst keratinization probably proceeds more slowly than normal, as indicated by the prominence of the granular layer. The epithelium sends out long pointed processes into the corium, and between these are the elongated, narrowed papillæ, which show some tendency to branching.

2. *Condyloma Acuminatum.*—In these akanthomas, which occur, for the most part, around the external genitals and anus, the horny layer is not at all, or very slightly, increased, whilst the greatly thickened prickle layer presents a mulberry or cauliflower appearance, owing to the continued branching of the narrow papillæ throughout their entire length, the branching being much more developed than in verruca. Mitoses are frequent, not only in the basal or germinal layer, but also in the three or four deepest layers of the prickle cells. The main differences between verruca vulgaris and these may be tabulated as follows:

Verruca Vulgaris	Condyloma.
Gross appearance: Flattened or semicircular nodule.	Cauliflower growth.
Gross appearance: Surface dry.	Surface moist.
Microscopical: Thickening of horny layer.	No thickening of horny layer.
Microscopical: Moderate branching of papillæ.	Excessive branching of papillæ.
Microscopical: Mitoses only in germinal layer.	Mitoses in germinal and also in three or four rows of prickle layer.

Vollmer thinks that in condylomas the primary growth is due to increase of the papillæ, but this is contested by Herxheimer, who, on examination of 30 specimens, was unable to find the necessary conditions for supposing this to be the case, namely, papillæ free from an epithelial covering. Herxheimer's view is certainly more in accordance with modern ideas, and may be accepted as in all probability correct.

Plate II.*a* shows a condyloma of the anus.

The flat condylomas are of different nature, the tumor being due

primarily to syphilitic lesions of the corium, whilst the thickening of the epidermis occurs as a secondary manifestation.

3. *Molluscum Contagiosum.*—These tumors appear as small, multiple nodules, usually the size of a pin-head to that of a pea, over which the epidermis rises up all round, whilst toward the center it sinks in, and, spreading out beneath the surface, forms a pouch or button of epithelial cells, with a central crater-like opening.

The cells forming the deepest layer are columnar, and rest on a basement membrane, which at more or less regular intervals runs up among the cells, giving to the pouch a lobulated appearance. Above the columnar layer are prickle cells, which quickly swell up and degenerate, forming large hyalin, oval bodies in the stratum granulosum, which, above this, become imbedded in a horny layer, being finally cast off with the horny scales through the central opening. There is a difference of opinion as to whether molluscum contagiosum takes its origin from the hair follicles or the lower layers of the epidermis, but all authors are agreed that the new growth is practically confined to the epithelium, and that the increase of connective tissue is insignificant.

In view of the admittedly contagious nature of these tumors, which is discussed at length by Stelwagen, 1805, the question of their parasitic origin has always naturally been prominent, and Neisser, among others, described the hyalin ovoid bodies as psorosperms, claiming, at the same time that these were the cause of the epithelial proliferation. For a time this view was held to be correct, but is now somewhat discredited, Unna and most other writers on the subject holding to the more probable theory that the ovoid bodies are degenerated cells. Bacteriological examination has always proved negative, and the parasite, if it exists, has yet to be discovered.

Plate I.*c*. Specimen brought from Vienna by Dr. O. H. Schultze; section through an entire pouch. The enlargement is not sufficient to resolve the individual hyaline bodies clearly, but under a higher power they can be seen imbedded in the deeply staining horny layer.

4. *Akanthoma Adenoides Cysticum of Unna, or Benign Epithelioma of Fordyce.*—Neither of these titles is quite satisfactory, since the word epithelioma is best reserved for malignant tumors arising from stratified epithelium, whilst Unna's akanthoma is misleading, for the cells of these tumors, although arising from the germinal layer of the epidermis, show no tendency to develop into prickle cells, and the growths should not, strictly speaking, be included among the akanthomas.

Fordyce suggests that certain cells of the germinal layer are destined to become prickle cells, and others to form the glandular ap-

pendages, so it may be supposed that it is from these latter that the tubular epitheliomas or rodent ulcers, and the benign epitheliomas, neither of which proceed to the formation of prickle cells, arise. According to this theory, the title adenoma cytadenogenes cysticum (cystic adenoma of the gland-forming cells) would perhaps be a good one to explain the nature of the growths, and but little more terrifying to the unfortunate possessors of them than Unna's. Jarisch calls these tumors tricho-epithelioma, believing them to arise from the hair follicles, but it is by no means certain that this is always the case. They occur, for the most part, on the open parts of the face, about the nose or orbit, and their location is a guide to their nature, since the syringadenomas, with which they are most easily confounded, are, as a rule, found on the trunk below the clavicle. They are said to be congenital, and at any rate are usually observed first in early life in the form of small pimples or nodules, which persist without further increase, for a number of years, though ultimately liable to become malignant, as in White's case, and the one figured here, Plate II.*a*, which started from a pimple of the orbit. Although a malignant growth, the photograph of it is inserted by way of illustration, since in its histological structure it is precisely similar to those which are still in the benign stage. In another case, examined at the General Memorial Hospital, the tumor originated at the orifice of Steno's duct, and after running an indolent course for over twenty years, developed malignancy, finally infiltrating the lymph nodes in its vicinity, the infiltration showing exactly the same structure as the original tumor. Dr. James C. Johnston suggested that this case be mentioned here, as he believes it to be a unique instance of infiltration of the lymph nodes. On microscopical examination these tumors are found to contain a somewhat delicate branching connective tissue stroma, on which rests a layer of columnar cells. In the distended alveoli are numbers of small cysts, filled with colloid material, and separated from each other by strands of cubical or flattened epithelium, the whole presenting the appearance of a net or lattice-work.

In addition to the usual forms of akanthomas, others are occasionally met with which cannot very well be classified. A few cases have been described of multiple hard nodules of the scalp, which microscopically are found to consist of closely packed, horny epithelial pearls, separated from each other by a scanty connective tissue stroma. Such tumors have sometimes been erroneously reported as endotheliomas. Plate III.*b* (Dr. O. H. Schultze) shows a photograph of a congenital pigmented wart, peculiar inasmuch as the pigment is entirely confined

to the germinal layer, none being found either in the prickle layers or the corium.

Tumors Arising from the Epithelial Appendages of the Skin.—Three varieties may be distinguished:

	Origin.
1. Syringadenomas.	Ducts of sweat glands.
2. Spiradenomas.	Sweat glands.
3. Steatadenomas.	Sebaceous glands.

Akanthomas may arise from the hair-follicles, but these cannot be said to fall into a group by themselves.

1. *Syringadenomas* (syrinx, pipe) arise from the ducts of sweat glands, and appear usually on the ventral aspect of the trunk, below the clavicle, as small nodules up to the size of a pea, although they may exceptionally reach a large size. Sections under the microscope present the appearance of small cysts, varying in size, containing granular and mucoid débris, and lined with cubical or flattened epithelium, in one or more layers, the cells swelling up with secretion of mucus, whilst at a later stage the epithelial lining disappears, owing to pressure. Török could find no connection between the cysts and normally formed ducts or coil glands, so concluded that the tumors arise from abortive ducts, the growth of which has become arrested during development, and which subsequently start in to grow atypically.

Petersen describes one in which he arrived at the same conclusions at first, but in a later article explains that he found connections between the normal ducts and those in the tumor. In the accompanying illustration the somewhat atrophied glands can be seen, whilst leading from them are the greatly distended and branching ducts which form the main part of the tumor (Plate III.*a*, case of Dr. Otto Schultze).

Spiradenoma.—True adenomas of the sweat glands must be distinguished from mere hypertrophy. Cases of the latter have been reported by Elliot and Beier as occurring in the neighborhood of irritated nævi, while Unna remarks that this condition is not infrequent in tuberculosis and in the neighborhood of malignant tumors. Plate IV.*a* represents its occurrence near the site of a carcinoma of the breast in a negress. Groups of hypertrophied sweat glands can be seen in the connective tissue of the corium, and below these is a distended milk duct.

There are usually a large number of glands affected independently of each other, preserving their normal form, but exhibiting greatly distended lumina lined with strikingly large epithelium, although the ducts are not necessarily closed. In neither Elliot's, Beier's, or the

case figured here was there any evidence of closure. Elliot supposes that the distension is due to the degeneration and swelling of the epithelial cells, the semi-fluid débris of which being so much thickened, remains in the alveoli instead of escaping by the ducts, and leads to the cystic formation.

True adenomas are more localized, originating probably from a single coil gland, though others in the neighborhood may become incorporated in the growth and possibly later add to its bulk by proliferation also. Still there is always a definite nodule formed by successive budding out from the affected gland of solid cell processes, which quickly show a tendency to assume coiled forms, and which may, or may not, acquire a lumen later (Plate IV.*b*). A scanty connective tissue stroma penetrates between the coils, whilst the basement membrane, on which the lowest layer of epithelial cells rests, is everywhere intact and may become greatly thickened and hyalin, as shown in the figure, Plate IV.*c*. Not many such cases have been reported, and very few of them are of the Cæsar's wife description, though they might, perhaps, all be good enough for Cæsar. Unna, 1894, accepts as genuine Thierfelder's, Hoggan's, Chandelux's, and Audry's. Chandelux's (1882) drawings and Audry's (1892) descriptions exactly tally with the appearance of the spiradenoma figured here (Plate IV.*b* and *c*, Dr. Otto Schultze), but Hoggan's (1881) illustrations show what is probably an endothelioma, whilst Thierfelder's (1870), to judge from his plates, has evidently reached a malignant stage, although undoubtedly originally a spiradenoma.

Fordyce (1895) describes minutely with excellent photo-micrographs an adeno-carcinoma of the coil glands, which probably originated as a benign growth. In his case there was distinct infiltration of the neighboring tissues, and in places the cells were observed breaking through the basement membrane.

More recently other cases have been reported: a very typical one by Spiegler, which, however, he calls an endothelioma, but his illustrations show the coils and solid budding processes so clearly that there can be little doubt but that he was mistaken as to its nature.

Steatadenoma.—The sebaceous glands are liable to vary physiologically in size and activity within very wide limits, a remarkable instance of excessive hypertrophy being found in the uropygeal gland of some birds, an enormous sebaceous gland situated at the base of the tail, the secretion of which is used in preening the feathers (Plate V.*a*). In consequence of this variability, the sebaceous glands are very apt to become hypertrophied under pathological conditions, and cause more or less diffuse swellings, as in rhinophyma and similar affections.

many glands enlarging independently of each other, but preserving their normal form (Plate V.*b*, from a case of rhinophyma). Such hypertrophies are often called adenomas, but cannot, strictly speaking, be regarded as such.

Again, many tumors supposed to arise from sebaceous glands develop prickle cells and horny material, so that they must be looked upon as akanthomas, which have simply invaded the glands, the latter taking no actual part in the tumor formation.

After exclusion of such cases, the number of genuine steatadenomas on record is greatly reduced; in fact, Unna, in 1894, considered that there was but one such, that of Bock, whose drawings and description certainly seem to be conclusive. Pollitzer, reporting a case in 1893, the first, according to him, in America, remarks that only about twenty cases altogether have been described up to that time, he including many which Unna rejects. It appears then that these tumors are, at any rate, very uncommon, and, taking the word steatadenoma in its most restricted sense, can hardly be considered to occur at all.[4]

Endotheliomas in the cutis, as elsewhere, rarely arise from the endothelium of the blood vessels. Wolters reports such a case, and gives very minute descriptions, with excellent drawings, of the microscopical appearance, calling it hemangio-endothelioma tuberosum multiplex, a name suggested by Jarisch, who had previously described a similar affection. Wolters identifies with his case many of those which have been described as lymphendotheliomas, syringadenomas (Török's amnog others), hidrocystadenomas, etc.

The patient, male, aged 30, had hundreds of nodules and spots up to the size of a pea scattered over the breast. The nodules had existed for a long time, and were probably congenital.

Sections showed the epithelial covering normal, whilst in the corium the stratum reticulare and subpapillare were thickly crossed with solid cell strands, swelling out at intervals into minute oval or round cysts, containing a central lumen filled with a plug of colloid material and lined with several layers of cells. These strands of cells could be traced arising from proliferating endothelium of the blood vessels, whilst that of the lymph spaces was everywhere normal.

Cases are more frequently reported as arising from the endothelium of the lymph channels, although most of these could probably be eliminated, as in reality epidermal.

Aschoff and Gaylord, for instance, figure one which gives the impression of an akanthoma adenoides cysticum rather than an endo-

[4] Since writing the above Dr. James E. Johnston has come across a specimen of an undoubted steatadenoma, a description of which, with photomicrographs he intends to publish before long.

theloma. Mulert describes an endothelioma of the scalp, but Lubarsch (*b*), after careful examination, affords what appears to be conclusive evidence that it was of epidermal origin, since he was able to trace the continuity between the tumor cells and the germinal layer of the epidermis in a few of his serial sections. Mulert's illustration, like that of Aschoff and Gaylord, is suggestive of an akanthoma adenoides cysticum.

When once the lymphendotheliomas become malignant, as not infrequently happens, it is difficult to distinguish between them and the carcinomas, but in their benign stages they can readily be recognized.

The endothelium swells up, forming cubical or polyhedral cells, which, by proliferation, give rise to other similar ones, distending and filling up the lymph channels, which now present the appearance of solid strands of epithelial cells, lying irregularly parallel with each other, the intervening fibrous tissue becoming greatly thinned out on account of the pressure.

In the case figured here (Plate V.c) the tumor, situated on the back of an adult, was congenital and had lately begun to increase in size. When excised, it had reached the size of a hazelnut.

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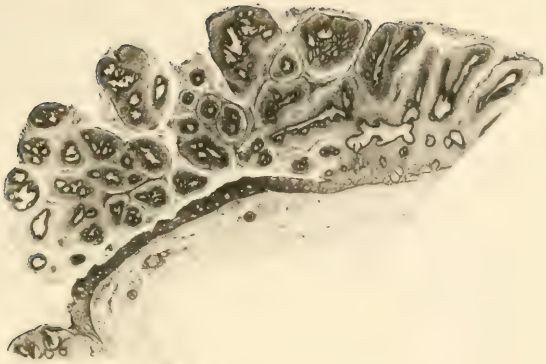

a.

b.

a. Carcinoma. X

b. Akanthoma adenoides cystic um. X

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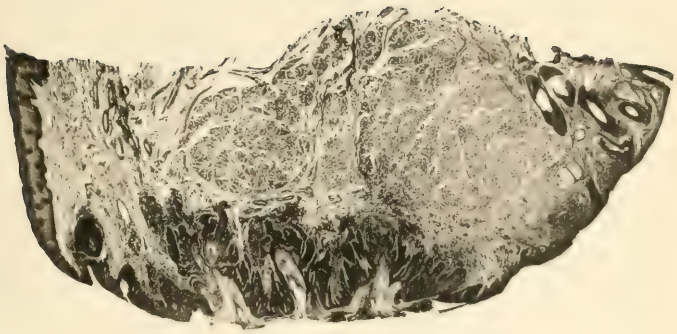
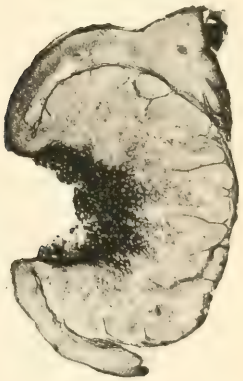


a.

b.



c.

a. Verruca vulgaris. X 15.
b. Keratoma. X 7.
c. Molluscum contagiosum. X 25.

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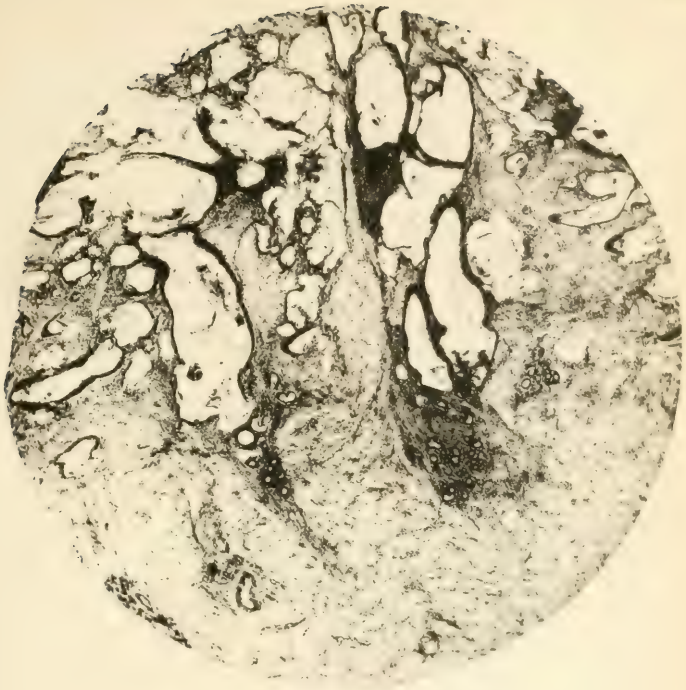
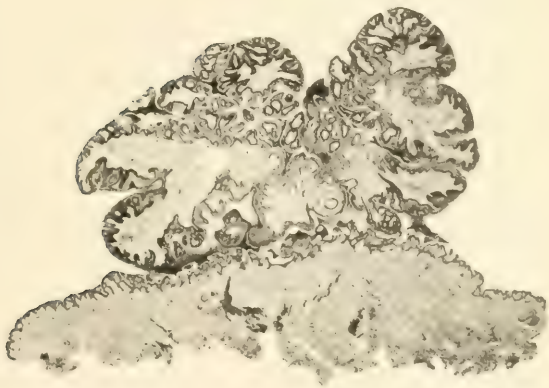


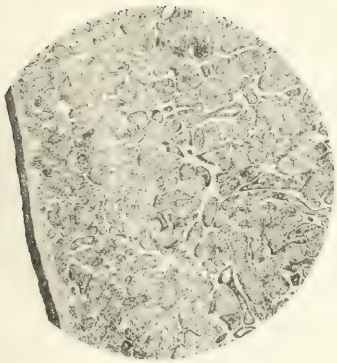
a.



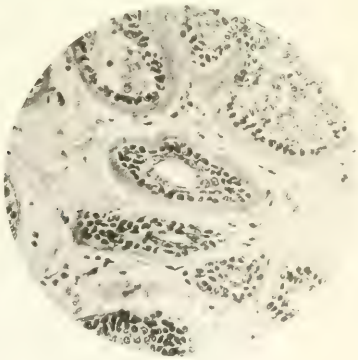
b.

a. Sarcoadenoma. X 25.
b. Pigmented wart. X 5.

A.



B.



C.

A. Hyperplasia of sweat glands. X

B. Spindle cells. X

C. X

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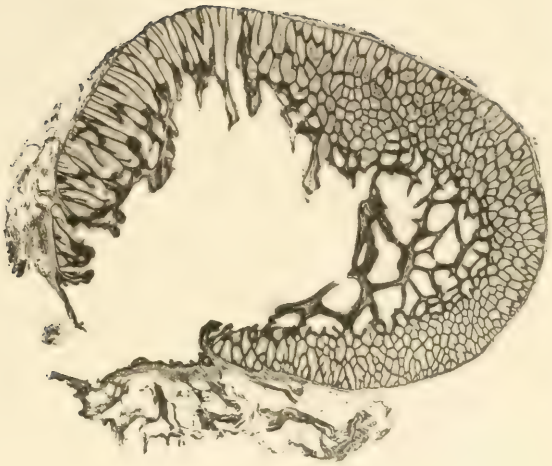
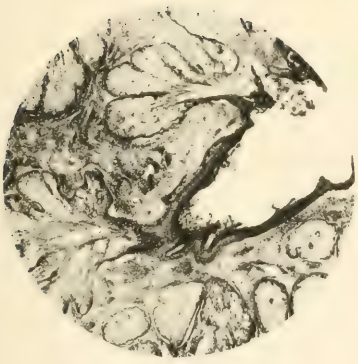
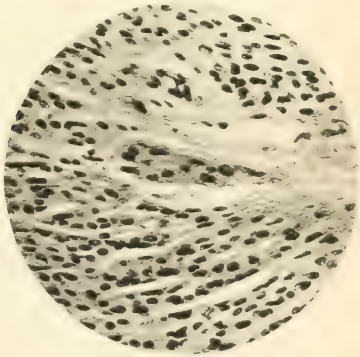


a.





a. Unpigmented gland of [illegible] X [illegible]
b. Hypertrophy of [illegible] glands X [illegible]
c. Endothelioma. X 250.

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A TUMOR OF THE KIDNEY ARISING FROM A SUPRARENAL REST.

By B. H. Buxton, M.D.

SINCE Grawitz[4] sixteen years ago first classified these tumors under the name of "Struma lipomatosa Suprarenalis," and pointed out their origin, numerous cases have been described and in spite of some opposition, notably by Sudeck,[13] who calls them "renal adenomas," and Driessen,[2] who calls them "endotheliomas," Grawitz's views are now generally recognized as correct. The cumbrous title adopted by him has been discarded and the word "hypernephroma," as suggested by Birch-Hirschfeld, appears to be the most suitable.

Aberrant fragments of suprarenals are not at all uncommon, being found usually in the neighborhood of the suprarenal vessels, and under the capsule of the kidney. They have also been described as occurring in the liver and broad ligament, etc. Their size varies from that of a pinhead to a pea. In structure they cannot be distinguished from the suprarenals, and almost, if not invariably, contain cortical tissue alone. Such rests may remain unchanged throughout life, or in course of time may take on pathological characters and develop into tumors. As a rule, this does not occur until late in life, the patients having been over 50 years of age in most of the cases described. The existence of these rests may be explained by the fact that the suprarenals are segmental in their origin, and the various foci may fail to coalesce during development.

The earlier embryologists taught that the suprarenals are of mesenchymal origin, but Weldon[12] in 1885 attempted to show for *Lacerta* and the chick that they are derived from the Wolffian bodies. Later investigators, Rabl,[9] Semon,[11] and others claim that they originate from the pronephros, remnants of which can be traced, even in mammals, at a very early period of embryonic life. That they are independent of the Wolffian body would seem to be confirmed by the fact that

in unilateral aplasia of the kidney and genital ducts, the suprarenal is almost invariably normal. Such cases point to a complete absence of the Wolffian body and duct, and that these arise quite independently of the pronephros seems to have been conclusively proved by Rückert[7] and Mollier.[8]

I have recently come across instances in two doe rabbits from the same litter, in which, in one case on the right and in the other on the left side, there was complete absence of the kidney, uterus, and Fallopian tube. The suprarenals were normal, and the ovaries and fimbriæ were present, but the duct from the latter ended blindly almost immediately. In short, the structures arising from the columnar epithelium and presumably the pronephros were present, whilst those arising from the Wolffian body and duct and the Müllerian duct were absent. Whether derived from the Wolffian body or pronephros, however, the suprarenals would be segmental organs originally, and the occasional scattering of the tissue can thus be accounted for.

So far as the kidney is concerned, the enclosed nests having once developed into tumors may reach a large size. Manasse[5] describes one the size of a man's head. They may grow slowly for years and then suddenly increase rapidly, or they may grow rapidly from the time they are first noticed. In the earlier stages they are usually benign in character, but later on, as a rule, develop malignancy, forming metastases in the liver, lungs, and other organs. They occur as nodular growths of firm consistence, surrounded by a definite capsule of connective tissue, from which trabeculæ proceed into the interior, forming a network, the meshes of which contain columns of cells supported by a fine delicate stroma, consisting almost entirely of capillaries and small veins, on the endothelium of which the cells often rest. As McWeeney[6] remarks, it is "stroma reduced to its lowest terms." The cells resemble those of the suprarenal cortex: the resemblance, as one would expect, being more marked in the smaller than in the larger tumors. The cells often contain fat globules and numerous granules of glycogen. They are fairly large, cylindrical, or polygonal, with a relatively small nucleus, which takes the usual stains readily.

These tumors are generally classed with the sarcomas, chiefly on account of the intimate relations between the cells and the capillaries, but, granting that the cortical portion of the suprarenal is derived from the pro- or mesonephros, they must be epithelial (mesothelial) in character, and from the anatomical standpoint can only be regarded as carcinomas. Graupner[4] and Lubarsch[5] suggest that, for the present, this be left open.

The following is the case in question:

Case.—Patient, H. J. L., male, bookkeeper, æt sixty-seven; admitted to the New York Cancer Hospital, January 5, 1898. Service of Dr. George W. Jarman, to whom I am indebted for the following history.

Ten years ago was operated on for fistula in ano; otherwise always in good health.

Supposed cause of disease:—None, unless holding water, which he did a good deal while at work. Noticed three years ago that water did not pass freely, but no other symptoms until nine months ago, when he complained of "swelling of the bowels," and a doctor discovered enlargement of right kidney. Has passed blood, once two years ago and again three weeks ago. Has lost flesh and strength and has a constant pain in the back.

Fig. 1.—Relations of kidney and tumor. (x 25.)

No operation; died February 21, 1898.

Autopsy, *February 22*, 1898.—Dead, twelve hours; condition emaciated; rigor mortis present; brain not examined.

Thorax.—Heart and lungs practically normal.

Abdomen.—*Intestines* normal. *Stomach.*—Pyloric end adherent to a nodule in the liver. Walls of pylorus thickened and infiltrated by continuity from nodule in liver. *Spleen and pancreas.*—Normal. *Liver.*—Congested and cirrhotic. On the under side is a nodule about the size of a small orange, which appears to have destroyed the gall bladder, and is adherent to the pyloric end of the stomach. The tumor has no definite capsule and is of firm consistence, except centrally, where it is broken down into a cheesy friable mass. *Kidneys.*—Left, enlarged, chronic parenchymatous nephritis; pelvis contains some pus. Right, converted into a

large sarcomatous mass. *Bladder.*—Enormously distended and filled with dark-colored urine, fairly clear, with an ammoniacal odor.

Gross Appearance of Tumor.—Oval, of firm consistence, studded with nodules of various sizes. It is completely encapsulated, and except for a few fibrous adhesions shells out easily from the surrounding tissues.

Weight, about 1000 grms. Length, 14 cms. Breadth, 10 cms. Circumference, long axis, 38 cms.; short axis, 30 cms.

On section the greater part of the contents is found to consist of friable degenerated material of a yellowish color; the lower part of the tumor alone appearing to be in an active growing condition. A capsule surrounds the whole; thick below, thinning out above, where it encloses the degenerated portions. From the capsule it extends into the interior trabeculæ, forming a network, in the meshes

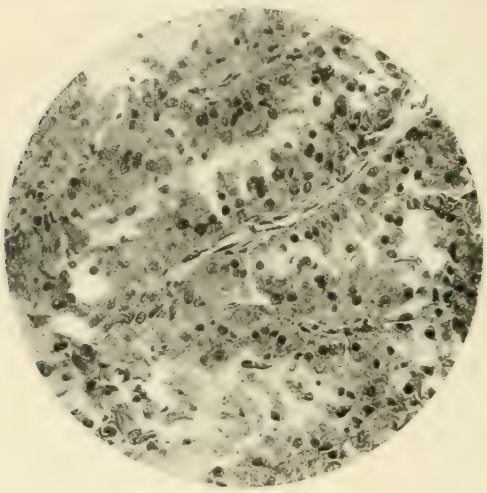

Fig. 2.—Rows of active tumor cells resting on collapsed capillaries. (x 200.)

of which are nests of tumor tissue, but this arrangement can only be observed in the actively growing areas.

Microscopically the capsule surrounding the lower part of the tumor is seen to consist chiefly of kidney tissue, compressed and more or less degenerated. Where the capsule is thinned out, the kidney structure cannot be traced. The tumor itself is separated from the remnants of the kidney by a thin capsule of dense connective tissue, containing few but well-organized vessels, and the trabeculæ running from this into the interior, and forming septa between masses of tumor cells, are similar in character. Here and there, shut in by the septa but

distinct from them, are remnants of kidney tissue containing degenerated Malpighian bodies with deposits of pigment.

The stroma of the parenchyma consists of thin-walled capillaries, some greatly distended, others collapsed, on which rest the cells, mostly in a single layer, forming long cylindrical ridges, which roughly resemble those of the zona fasciculata of the suprarenal (Fig. 2).

The cells themselves are large, cylindrical, or polygonal, with a small round nucleus staining deeply with hematoxylin. Degeneration, where the cells are numerous and the capillaries for the most part collapsed, seems to proceed rapidly by a process of coagulation necrosis, but in other parts of the tumor, where the capillaries are greatly distended and the cells relatively few in number, hyaline degeneration of the vessel walls with fibrinous coagulation of the contents is a marked feature, and the general appearance that of an angio-sarcoma (cylindroma). In many places the cells contain fat globules.

The metastatic nodule in the liver and infiltration of the pylorus resemble each other for the most part microscopically, but differ from the primary tumor in the size of the cells, which are much smaller, with more irregularly shaped nuclei. The grouping of the cells around the capillaries is also more irregular than in the primary tumor, though on the whole it bears a general resemblance to it.

Just beneath the mucous membrane of the pylorus, and limited to this, are numerous small areas, mostly about the size of a pinhead, of colloid degeneration.

The question arises as to whether the nodule in the liver is metastatic or a second primary tumor. Structurally, it certainly resembles a sarcoma rather than a carcinoma, whilst the colloid degeneration of the cells in the pylorus would seem to point to their epithelial origin. In this it corresponds to the synopses in descriptions of hypernephromas: "Sarcomas from the morphological, carcinomas from the anatomical standpoint."

The autopsy had to be somewhat hurriedly performed, and, the significance of the tumor not being recognized at the time, examination of the suprarenals was, most unfortunately, neglected, so that the possibility of the tumor having arisen from the suprarenal itself and not from an enclosed rest must be conceded. But, if the former had been the case, the kidney would probably have been merely pushed aside and not invaded, so as to form a capsule to the tumor with fragments of it remaining in the septa. Besides, the position of the tumor corresponded exactly to that of the kidney, the extension above being about the same as the extension below the normal position. The occurrence of hæmaturia also points to a tumor of the kidney.

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An Improved Photo-Micrographic Apparatus.

I have recently had constructed for Cornell Medical College a photo-micrographic apparatus by B. & L., a description of which may be of interest to the readers of the JOURNAL, since there are certain departures from the regular type of outfit. Some of these departures were suggested by me, but they were worked out in detail and put into practical shape by the makers. To begin with, I will point out the various alterations, all of which, I think, are improvements over the old style, and afterwards proceed to describe the apparatus as a whole.

In the first place, then, on the old plan the optical bench and camera stand being practically all in one piece, the only way of finding the desired field for photographing is to push the camera back in its bed and bend over to look through the eye-piece. In this uncomfortable and back-breaking position it is impossible to manipulate the slide except by means of one of those clumsy mechanical stages which, though all very well for special purposes such as blood cell counting, are not to be compared with one's fingers, which, after all, were made at a much earlier date, for rapid manipulation.

In a Zeiss camera stand which I had previously used, the steel rods on which the camera moved could themselves be pushed back so that one could sit on a stool between the optical bench and camera stand in order to find the field. The chief objections to this plan are that the rods are apt to sag, and that the bench and stand cannot be connected into one solid piece, making it difficult to preserve the exact optical axis, or arrange a satisfactory method for mechanical focusing.

Two years ago I saw at the Jenner Institute in London a photo-micrographic outfit with a revolving optical bench, but had no opportunity of examining the details or of finding out if the arrangement worked satisfactorily or not, since it was entirely new. On mentioning the idea to Bausch & Lomb, they suggested a modification of one of their revolving microscopical tables. The details will be described further on; sufficient to say here, that by this arrangement I can sit comfortably at one side of the apparatus and with my fingers manipulate the slide on the microscope stage as easily as if I were sitting at an ordinary table.

The camera stand has a connecting rod between its two cast iron supports, just above their feet, and I then suggested that the rod be continued on to the single upright of the revolving bench in order to give more rigidity, but was told that so low down it would be of no use. After much discussion, it was decided to have a rod connecting the upper parts of the uprights, and so made that it could be put up and clamped in at the time of setting up the apparatus. This seems a very satisfactory arrangement, undoubtedly adding to the rigidity and helping to keep the optical bench and camera stand in optical axis.

The next difficulty was how to manage for mechanical focusing. When the bench and stand are fixed there is, of course, no trouble, since the focusing rod can be prolonged under the fine adjustment of the microscope, but where the bench revolves this prolongation of the rod must be got out of the way tempora-

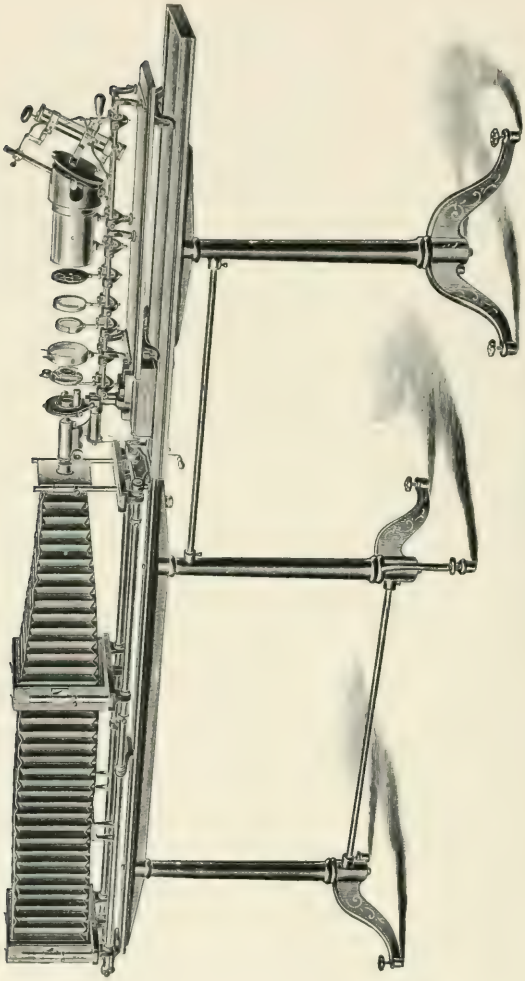


Fig. 1.—Complete Photo-micrographic Apparatus.

rily while the field is being found. Mr. Bausch asked me how I proposed to effect this, and I was forced to reply that I had not the least idea, but must leave it to him. Mr. Patterson, who had charge of the work, came to the rescue with a very ingenious device, which will be described further on.

It is, however, always best to avoid mechanical focusing, and be able to reach the fine adjustment with one's fingers, if possible, especially for the higher powers. In the B. & L. stands that I had hitherto seen, this appeared to be an impossibility, except within a very limited range, since the arrangement of the bellows was such that a shorter distance than fourteen inches could not be obtained between the eye-piece of the microscope and the ground glass of the camera.

Besides this, the front of the camera was always made the same size as the back, i. e., 11 inches square for a $6\frac{1}{2} \times 8\frac{1}{2}$ plate, so that the wrist had to be bent round over the front to reach the fine adjustment, thus losing three or four inches of distance for hand focusing.

Not only is the hand focusing superior in accuray, but it is essential for another reason to be able to get the ground glass close up to the front, since the up-to-date apochromatic lenses will stand very high eye-piecing, with consequent shortening of focus. The higher the eye-piecing the shorter the focus for a given enlargement, as is illustrated by the accompanying photographs, all of which were made with this apparatus.

It will be noticed from the description and the accompanying cut that the disadvantages mentioned have been overcome. With the new apparatus I can bring the ground glass as near as seven inches from the eye-piece, and can focus by hand without any flexing of the wrist, the total range for hand focusing being about sixteen inches with a Zeiss projection ocular. The choice of a microscope stand was narrowed down to a Zeiss photographic and a Bausch & Lomb DD, the latter of which was finally decided upon since the former does not appear to offer any very special advantages, and the fine adjustment is situated so far forward that two inches in length would be lost for hand focusing.

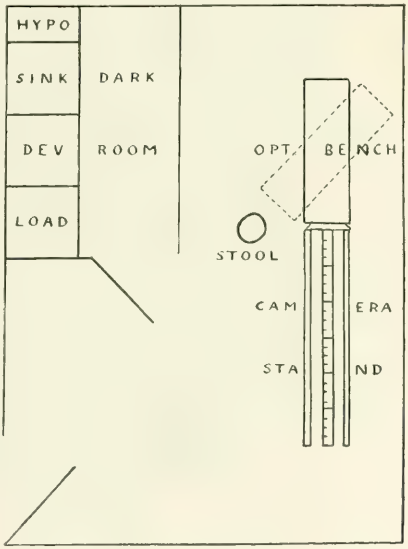


Fig. 2.—Plan of Room.

Another improvement suggested was in making the camera stand entirely of metal. The wooden bed of the old style is liable to warp, and thus throw the rods out of true.

It would have been an advantage to have had one of the rods marked off in quarter inches, so that once the length of bellows for a certain enlargement had been accurately measured, the back of the camera could at any time be brought to the same position without any delay in measuring. This point was overlooked, but I have had quarter-inch spaces marked in white paint on the flat surface of the upper connecting iron casting, and this answers the purpose just as well. (See plan of room, Fig. 2.)

The optical bench carrying lamp, microscope, and accessories is 4 feet by 15 inches and is a turntable, revolving upon the supporting column, so that it can be turned partially round; the operator sitting on a low stool to find the field and rough focus.

Since the dark room is on the left of the apparatus, looking towards the source of illumination, the table has been constructed to turn to the left, and everything else is so arranged that it can be manipulated from the left, thus obviating any necessity for walking around the apparatus.

The accompanying diagram illustrates the plan of the room, 11 x 15 feet, the dotted lines showing the optical bench in position for finding the field. Of the camera stand only the steel rods on which the camera runs, and the central beam with some of the markings, are indicated. The lamp and accessories run on steel rods, which are supported on a wooden table, supported in its turn on leveling screws, which run in a metal groove on the surface of the optical bench, so that the table can be moved backward or forward as required.

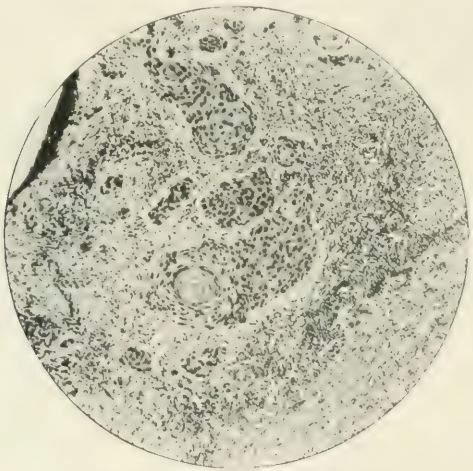


Fig. 3– Epithelioma invading lymph node. x 70. Lens, Powell & Leland 1 in. apochromatic; ocular, none; exposure, $\frac{1}{2}$ sec.; distance of plate from hood, 70 inches.

The rheostat is a Colt adjustable, and stands on the floor just below the illuminating end of the optical bench. I had previously used an automatic arc

1370 lamp, but finding that as a rule I had to be my own automaton, decided that a hand feed lamp would do better, since less liable to get out of order, and so far have no reason to regret the change.

The condenser and accessories are the regular Bausch & Lomb, so need no special description. In the figure the order from the lamp is: (1) condenser, (2) iris diaphragm, (3) paralleliser, (4) ray filter, (5) water tank, (6) iris diaphragm shutter. In practice so far, however, I have dispensed with the iris diaphragm, paralleliser, and ray filter, putting the water tank next to the condenser, and between tank and shutter using a flat tray on which ground or colored glasses, or a glass trough containing Zeltnow's solution, can be placed, so that the ray filter can be changed at a moment's notice. The lamp and condenser are then arranged so that the latter focuses directly on the substage condenser

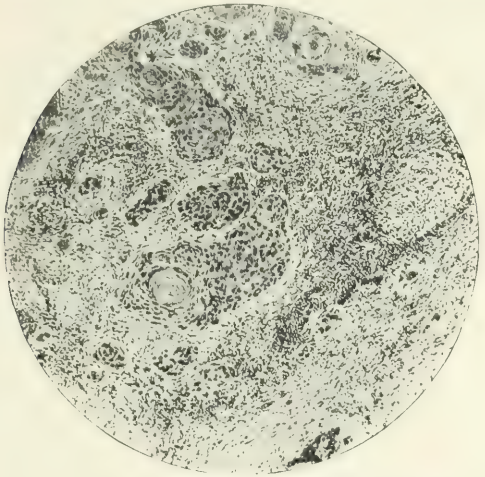


Fig. 4.—Same as Fig. 3. x70, Lens, B. & L. 1 in. apochromatic, ocular, B. & L. 1 in compensation; exposure, 1 sec; distance of plate from hood, 12 inches.

or on the slide if no substage condenser is used. By this means a tremendous flood of light is thrown on the object, and exposures can be cut down to a fraction of a second without, so far as I can judge, affecting the results for the worse. On the day of writing this, with an exposure of one-half second, I photographed a small round cell sarcoma 250 diameters on a Cramer slow isochromatic.

The microscope stand rests on a fixed wooden block to which its horseshoe foot can be clamped.

The camera stand, as seen by the cut, consists of two cast iron uprights, connected above by a solid cast iron beam on the top of which are the already mentioned one-quarter inch spaces marked in white paint. This beam supports the steel rods upon which the camera runs.

The front of the camera is five inches square, and has vertical and horizontal movements. The bellows is divided into two, being so constructed that the back part can be taken off the central wooden frame when a short focus is required, and pushed back out of the way. The ground glass and plate-holder can then be fitted into the central frame. Both the back and central frames have vertical and horizontal movements, and are precisely alike in every particular, so that either can be used for focusing and exposing.

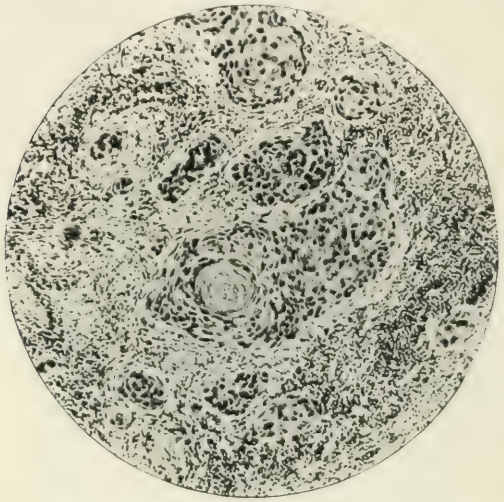


Fig. 5. Same as Figs. 3 and 4. x 100. Lens B. & L. 1 in. apochromatic; ocular, ½ inch; exposure, 2 sec.; distance of plate from hood, 7 inches.

With regard to the arrangements for locking the optical bench, and mechanical focusing, I append a short description.

In making the mechanical attachment of a photo-micrographic camera for the purpose of operating the fine adjustment of the microscope, two points must be considered, viz.:

First. An arrangement whereby the attachment can be operated from any position, and

Second. The operation of the microscope fine adjustment without lost motion or back lash.

In the camera described above, a third problem is presented, in that the optical bench carrying the microscope and illuminating apparatus is arranged to rotate upon the supporting column, enabling the operator to adjust the specimen, illumination, and preliminary focus before connecting the microscope with the camera.

It will be seen that this arrangement necessitates the detaching of the focus-

ing rod of the camera from the microscope. To avoid the displacing and replacing of the belt connecting the mechanism, the following arrangement is adopted:

On the table of the optical bench, directly beneath the fine adjustment head of the microscope, is situated a milled wheel on suitable standard. A belt extends from this wheel to the fine adjustment head of the microscope. Through the axis of the wheel is located a rod carrying at the end toward the camera a clutch which can be quickly connected with the focusing rod of the camera by sliding adjustment, operated by a milled head. The optical bench must of course be brought to its proper position with relation to the camera in order to make this connection, and, that this position may be quickly and accurately located, an automatic catch is provided, which catch can be released by a lever, shown in the illustration.

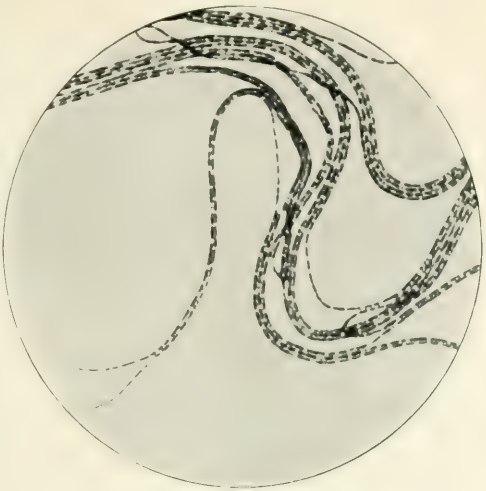
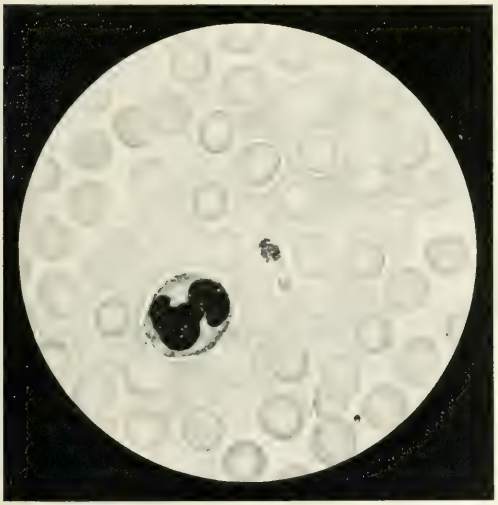


Fig. 6.—Anthrax, impression preparation from edge of colony on gelatin. x700. Lens, $\frac{1}{12}$ oil immersion; ocular 1 in. compensation; substage condenser, oil immersion N. A. 1.40.



Malarial Parasite in Human Blood. Cresentic form. x1200.

B. H. Buxton.
Cornell Medical College.

Reprinted from Annals of Surgery, September, 1901.

TERATOMA OF THE TESTIS.[1]

By WILLIAM B. COLEY, M.D.,

AND

BERTRAM H. BUXTON, M.D.,

OF NEW YORK.

The patient who is the subject of the present report was a German, aged twenty-seven years, who has always been in perfect health. He states that up to eight months prior to December, 1900, he had noticed nothing abnormal in the right testis, and he had never received an injury as far as he remembers. About this time he observed a slight enlargement of the testicle. This increased uniformly, without pain, until the time of my first observation, December 10, 1900. Physical examination then showed a tumor about the size of an orange, symmetrical in shape, very firm in consistence, giving all the characteristics of a sarcoma of the testis, which was the clinical diagnosis made. The glands of the groin were not enlarged and general health was perfect. On December 12, I operated, removing the testis and the cord up as far as the internal ring. (See Fig. 3.) The wound healed without suppuration and the patient has been in good health since.

Pathological Report on Tumor of Testis.—The tumor is about the size of an orange, the testis forming a flattened cap over the upper part. Both tumor and testis appear to be contained together in the tunica albuginea, although the former is enveloped by a separate capsule of connective tissue, from which the testis can be peeled off without tearing.

On section the tumor is found to consist chiefly of tough, semitranslucent material containing numerous minute cysts. (Figs. 2 and 3.) Microscopical examination shows a teratoma with all three germinal layers represented.

The *ectoderm* by cysts lined with flat, stratified granulosum and formation of horny material. Hair follicles and glandular appendages are absent.

The *mesoderm* by islands of hyaline cartilage with a tendency in a

[1] Read before the New York Surgical Society, March 27, 1901.

few places towards ossification: mucoid and spindle connective-tissue cells resembling those found in the embryo, and involuntary muscle fibres.

The *entoderm* by irregular, villous cysts lined with columnar epithelium and containing mucus. The larger cysts have lost their villosities, and the lining epithelium has become more or less flattened, presumably owing to pressure. The walls are composed of embryonic connective tissue interspersed with strands of involuntary muscle fibres.

In close connection with the entodermal cysts are masses of swollen hyaline cells, lying closely packed together in definite alveoli without any intercellular substance between them, and which may perhaps represent the chorda dorsalis. Wilms has observed these masses of cells in his cases, and supposes them to represent early stages of stratified epithelium, but their connection with the entodermal cysts seems to point to their being of a different nature. The entodermal cysts greatly exceed the ectodermal in number, and this condition was also observed by Wilms. And these different structures are heaped up together without any particular arrangement, so far as can be observed.

The tumor appears to be of a benign nature in that it is enclosed in a definite capsule, and the cells of which it is composed, although embryonic in character, seem to indicate progressive development rather than the regressive changes observed in carcinomas and sarcomas.

This case, though very rare, is interesting chiefly from a pathological stand-point, and a very careful report of the microscopical findings, illustrated by photographic reproductions of slides of the specimen, has been prepared by Dr. Buxton.

Wilms's paper (*Beiträge zur Pathologischen Anatomie*, Ziegler, Band xix) is undoubtedly the most complete and valuable work we have upon teratoid tumors of the testis, but we must remember that it was written entirely from the point of view of the pathologist.

The material upon which his paper is based comprises ten tumors of the testis observed by himself at the Pathological Institute of Giessen, and which he classes as "Mischgeschwülste des Hoden," and fifteen others, classed as dermoid cysts, that he has collected from literature. To show the confusion that has existed up to the present time, and still exists, as to the classification of these tumors, we need only to note that the original microscopical diagnosis in the ten cases which he classes as teratoid tumors was as follows:

The first case was classed as a myxosarcoma;

The second as a cystosarcoma;
The third as a carcinoma;
The fourth, mixed tumor of the testis;
The fifth, carcinoma;
The sixth, medullary carcinoma;
The seventh, cystoma;
The eighth, cystoid disease of the testis;
The ninth, cystosarcoma of the testis;
The tenth, teratoma, with malignant degeneration.

I do not think we should adhere too literally to the view of the pathologist, that these teratoid tumors are non-malignant. In two of Wilms's ten cases the subsequent history proved them to have been malignant, and in several of the remainder the after-history was not traced.

F. R. Sturgis, in 1899 (*American Medical Quarterly*), published a case of cystoid disease of testis, or a doubtful teratoma. The tumor was malignant, a carcinoma, and the patient died less than a year after. Sturgis has collected from the literature forty cases of cystoid disease and tabulated them. Of these forty cases, nine showed evidences of sarcoma; two of carcinoma, in the specimens removed. Sixteen, however, afterwards died of metastases; and, furthermore, the after-history of twenty-two others was not traced more than a few months after operation.

The conclusion must be that clinically these tumors are far more malignant than we are led to believe by the pathologists.

Teratomas may occasionally develop malignancy. Hudson, Welch's *Festschrift*, 1900, reports an adenocarcinoma arising from tracheal mucous glands in a sacral teratoma, and observes that this appears to be a unique case of glandular carcinoma in these tumors, since the few hitherto reported were of the squamous-celled variety. Wilms (*loc. cit.*), in ten specimens of teratoma of the testis, considered two to be clinically malignant; one because it formed secondary nodules in the neighborhood of the primary growth, and the other because secondary nodules developed in the liver some

time after operation on the primary tumor. Still, even in these two tumors there was no true infiltration of the neighboring tissues, such as there would be in carcinoma or sarcoma. So he concludes that histologically they would not be considered to show malignancy. Nevertheless, he admits that the tissues of teratomas may develop carcinomatous or sarcomatous degeneration, and for this reason should always be removed, even if there is no immediate danger on account of rapid growth.

Etiology of Teratomas or Embryomas.—Various theories have been advanced to account for their origin:

(1) Two ova are impregnated, one of which grows imperfectly and is incorporated by the other. This seems to account for acardiac parasites, and perhaps for those cases in which the tumor is found lying loose in the peritoneal cavity.

(2) Impregnation of one ovum with splitting off of one or more cells during segmentation before the establishment of the germ layers. Driesch showed in ascidians and echinoderms that in the two-cell stage of segmentation each cell, if separated from the other, is able to develop into a perfect embryo, which, however, is smaller than normal. Wilson in amphioxus, Hertwig and Schultze in amphibia, and others in various animals, have confirmed this, some observers showing that in the eight- and even the sixteen-cell stage a single separated segmentation cell is capable of producing an embryo normal in every respect save that of size.

Cephalic and sacral teratomas are ascribed to a similar process: the separated segmentation cells becoming enclosed in the process of enfolding of the medullary groove, and in later life producing a parasitic growth.

Next to the cephalic and sacral teratomas those of the ovary and testis are most frequent, but, in the present state of our knowledge of embryology, it is not easy to understand how separated segmentation cells can become incorporated in the genital organs, and in consequence the origin of these tumors has been ascribed by some authors to

Fig. 1.—*Teratoma of Testis.*—On the left are the tubules of the testis separated from the tumor itself by a band of connective tissue forming the capsule of the tumor.

In the tumor itself the dark areas are masses of cartilage. The cysts are seen in places encircled by a dark line. This dark line represents stratified epithelium, whilst those cysts in which it does not occur are lined by a single layer of cubical or columnar epithelium, the latter in some places ciliated.

The stroma, in which are scattered the cysts and islands of cartilage, is composed of embryonic-looking spindle-cells for the most part, whilst surrounding many of the cysts are layers of involuntary muscle fibres. Such cysts probably represent the intestinal tract, and their lining cells must be considered as of entodermal origin.

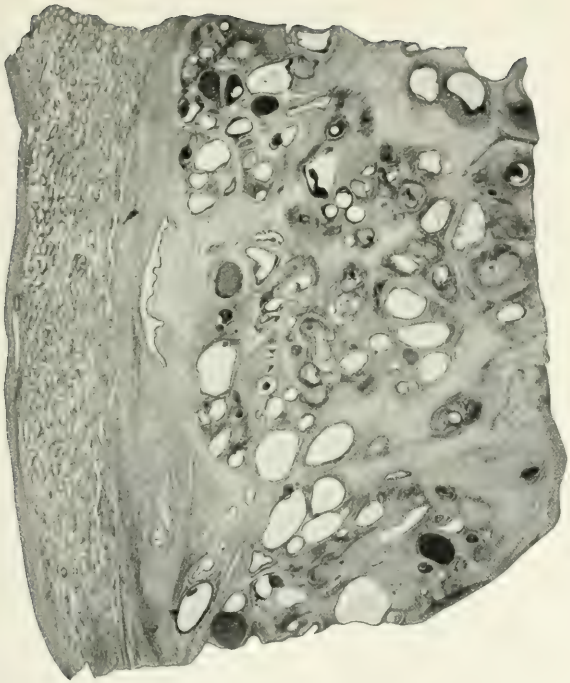


Fig. 2.—*Teratoma of Testis.*—Taken from the interior of the tumor.
The islands of cartilage (dark areas) and cysts are here larger than at the periphery of the tumor.
The connective-tissue stroma is more highly organized, as also are the bands of involuntary muscle fibres surrounding many of the cysts. These bands are visible in the print; staining somewhat deeper than the connective tissue.

(3) A partial hermaphrodism with consequent fertilization of an ovum in the genital organ itself. Wilms professes himself unwilling to speculate rashly on the origin of these tumors, but infers that, since all three of the germinal layers are represented, they must arise from an impregnated ovum. There are certain large cells lying in the interstitial tissues of the testis which Nussbaum supposed to be undeveloped primordial ova; but most observers believe them to be of connective-tissue origin; and Lockwood claims to have traced their development in the embryo from connective-tissue cells, while the fact that in those animals—boar, cat, etc.,—in which they are specially prominent, they vary considerably in different seasons of the year, points to their fulfilling certain unknown functions.

Wilms suggests that, in view of Nussbaum's opinion, if the origin of testicular teratomas could be traced to these cells, it might furnish a clue to the reasons for their formation. However, although he admits that it is difficult to be certain, he concludes from his observations that they do not arise from the interstitial tissues, but are of intracanalicular origin. This being the case, he considers that there is probably a partial hermaphrodism, and that an ovum is impregnated by a sperm cell of the testis.

This might possibly occur in the majority of the patients, since the tumors usually appear upon puberty; but it is inconceivable in the cases reported by Kockel (three years) and Lovett and Councilman (three months). In these two cases, at least, there can have been no auto-impregnation; and indeed Wilms himself seems to think that the tumors are usually congenital, becoming apparent in later life.

Bonnet (Merkel and Bonnet's *Ergebnisse,* 1899) points out that in cases of hermaphrodism, even in lower vertebrates, ripe sex cells of only one kind are found, those of the other kind, whichever it may be, remaining abortive.

Partial hermaphrodism therefore does not seem sufficient to account for the teratomas.

(4) Parthenogenetic fertilization of an ovum according

to Pfannenstiel and his followers. This might conceivably occur in the ovary of an adult, but would not account for those of congenital origin, nor could it occur in the testis. Since teratomas of the ovary and testis are precisely alike, it is probable that their origin, whatever it may be, is the same, so that a parthenogenetic origin may be ruled out.

(5) The same objections apply to the idea that fertilization of a polar body is the cause of such tumors in the ovary. This theory is based on the known fact that in certain invertebrates one of the polar bodies may become fertilized and develop into a perfect embryo. It seems necessary, therefore, to return to the separated segmentation-cell theory and endeavor to make it account for these growths. It has been shown, as already remarked, that single, dislocated segmentation cells, even up to the sixteen-cell stage of division, may produce perfect embryos; and it may therefore be assumed as probable that any single segmentation cell up to, or almost up to, the point of the formation of the germinal layers possesses the potentiality of producing all of those layers if occasion arises. If, then, such a segmentation cell be detached at the margin of the ovum, it would lie on the surface of those cells which are destined to form the ectoderm and entoderm, and would probably be cast off, though it might accidentally become included at any point where there was an infolding from the surface. Such an infolding occurs along the dorsal surface of the ectoderm to form the medullary canal; and here the cell might become enclosed and form a nucleus for a cephalic or sacral teratoma. Such an infolding occurs also along the entoderm to form the chorda dorsalis, and vertebral teratomas have been reported, although apparently no attempt has been made to decide if they arose in connection with the vertebræ themselves or with the medullary canal. This is a point which it would be difficult, if not impossible, to determine, even if the attempt were made.

If a segmentation cell near the centre of the ovum were detached from its fellows, it would have no chance of escape, but would remain included at a point where the mesoderm

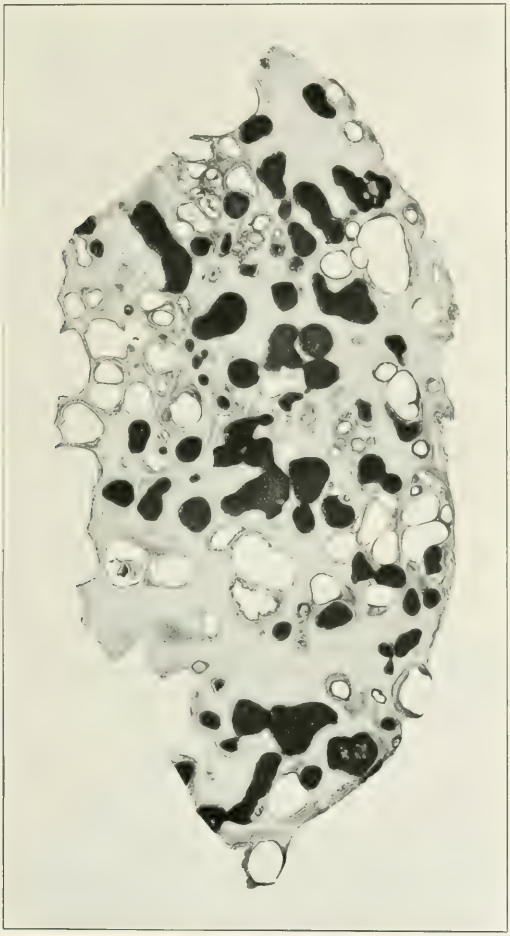


Fig. 3.—Teratoma of testis.

would probably first make its appearance. If such a cell remained entirely surrounded by the normally increasing tissues, it would probably soon become absorbed, but on the formation of the body-cavity might happen to fall into it and there remain alive, gathering strength and the possibility of an independent existence by drawing in the serous exudation for its nourishment. On the formation of the genital ridge the surface epithelium at this point becomes columnar, whereas, over the rest of the body-cavity it flattens out into endothelium. It might be supposed, then, that one segmentation cell would be more likely to become entangled in and enclosed by the columnar epithelium than the flattened, and thus be carried down into the ovary or testis, as the case might be.

A CASE OF MULTIPLE ANGIOSARCOMA OF THE SKIN.

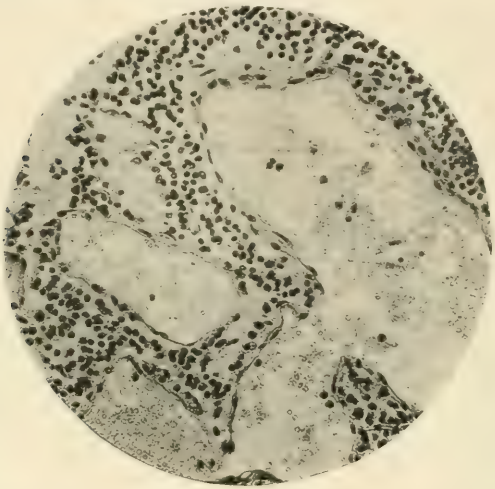
By James C. Johnston, A.B., M.D.,
New York.

THE case, which has some uncommon features, was turned over to me by Dr. F. W. Gwyer, of this city, from his service in Bellevue Hospital. The patient was a child, 16 months old, well nourished and in good health. The tumors, whose nature was unsuspected, were scattered in small number over the body, a departure from the general run, in which the growth is solitary and located on the head. The nodules were rather large, an inch or more in diameter, soft, movable, elevated, non-pigmented, and covered by a smooth epidermis. In cases of long standing, thickening of the horny layer is apt to occur. It is not possible to determine whether the multiple growth was due to metastasis, but it seems fair to assume, in view of their character and the age of the patient, that the tumors developed simultaneously. The history sheds no light on this point. There was no lymph-node involvement, and as nothing further has been heard of the case, the question of recurrence must remain unsettled. At the operation (October 29, 1899) the growths were found to be rather sharply limited and the clinical diagnosis of vascular nævi was made. The tumor process being endotheliomatous, benign in most instances, it is likely that excision effected a complete and permanent cure. I am aware that the history is meager, but it is my experience that atypical neoplasms in the skin are correctly diagnosed only, if at all, at the microscopist's table, and the histology is incomparably of greater importance.

Histology.—One tumor, from the shoulder, was sent to the laboratory of Cornell University Medical College. It was, before hardening, perhaps, in its greatest diameters, 1½ by 1 by 1 inch, and was shelled out of the surrounding connective tissue, making the exact point of origin difficult to determine. The new formation takes place apparently only in the blood capillaries; the lymph channels are not involved, for the new spaces contain blood in every instance. Lobulation is apparent even in the gross appearance, fibrous septa surrounding and separating neighboring tumor masses. This connective tissue is not a new development, but is a condensation resulting from new growth between preëxistent fibers. There is no evidence in the latter of proliferation. The limit of the neoplasmatic masses

below is the subcutaneous fat; consequently, it is probable that the capillaries of the reticular layer next the adipose tissue furnished the starting point of the new formation.

The lobules are made up of numerous dilated vessels, lined with swollen endothelium, a supporting reticulum of delicate areolar tissue and masses of cells. The last are arranged in concentric rows about the vessels, in islands and in strings, the individual elements placed end to end. The cells are round or cuboidal from pressure, with a clear protoplasm, having little affinity for dyes of any sort, condensed at



the periphery into a false cell membrane and a central, round, diffusely staining nucleus, in a word, embryonic endothelium. The same type is seen in other endotheliomas and in certain specific inflammations of low grade. My first impression was that the new vessels were formed before birth, lined by rather flattened cells, and that they proliferated externally, as they sometimes do in other endotheliomas. A further examination leads to a different interpretation. As in granulation tissue, the endothelium of the capillaries involved puts out solid buds of cells, through which the blood is forced, hollowing out a channel for itself in their center in some instances, in others finding its way

into a space formed by a process of vacuolation, also in the central portion. In either case, the new channel soon becomes lined by flattened cells, the embryonal bodies reassuming their special forms. The process of vacuolation (I cannot better describe it) can be seen in many of the separate islands, the space empty, since the blood has not yet found its way into them. Once formed, the new channels widen rapidly. The strings of cells can be explained by snaring off some portion of the growth by connective tissue, often seen in epithelial neoplasms. Proliferating, the spread is along the line of least resistance, in single-celled chains. The accompanying photograph, taken by Dr. B. H. Buxton, is from another case, but shows a condition identical with that I am describing. It is readily seen that this tumor is by no means identical with those described by Fordyce (*Amer. Journ. of Med. Sciences*, August, 1900) and Winfield (JOURN. OF CUT. AND G.-U. DIS., April, 1900.) In both instances, the term angiosarcoma is correctly applied, because, although there are new-formed vessels (blood and lymph channels in Winfield's case) cellular proliferation occurs in perivascular fibroblasts, and the cells are spindle shaped. While endothelium and connective tissue corpuscles are congeners, they do not in adult life assume each other's function, and tumors derived from the first are much more nearly allied to epithelial than to connective tissue neoplasms. If a precise terminology is a desirable thing (it seems doubtful in specific instances) the proper appellation in this instance would be hemangio-endothelioma.

Wolters has recently recounted two cases (*Archiv f. Derm. u. Syph.*, September, 1900, p. 269 with plates) which he calls hemangio-endothelioma and hemango-sarcoma. The first closely resembles this case, being multiple, the tumors brownish and flat or papular. Endothelial proliferation resulted in blocking the vessels, a feature absent here. Otherwise, the histology is identical. The second case belongs to the Fordyce-Winfield type.

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SARCOMA AND THE SARCOID GROWTHS OF THE SKIN.[1]

By JAMES C. JOHNSTON, A.B., M.D.,

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(From the Laboratory of Cornell University.)

THE object of this paper is not so much to present the results of original investigation, although the illustrative cases are unique in certain features, as to bring about, if possible, an agreement in the consideration of the subject of sarcoma in dermatological and general pathological literature. There is wide variance at present. Pathology takes no notice of the peculiar growths the skin presents, and dermatology ignores work outside its own field, so that the task is not easy. Literature on cutaneous sarcoma is more than abundant, authors' opinions are often diametrically opposed, and some of the cases reported are useless through indefinite wording or open to question from misconception of their nature. I have made no pretense of an encyclopedic review of works on this neoplasm.

The etiology of the entire class of sarcoma and its congeners is unknown. No advance in this regard has been made since the first member was described except that it is now fairly safe to deny a rôle to any micro-organism known. The statement that the sarcomatous process

[1] Read before the Buffalo Academy of Medicine and the American Dermatological Association.

is a perverted repair is only begging the question. The cells themselves are parasitic, and literally prey upon their host.

This basis of the classification used here is histogenetic solely. In the present state of knowledge, embryology furnishes no solid ground for any arrangement of tumors. On what seems reasonable premises I have divided sarcomatous neoplasms into three groups: (1) Fibroblastic (true) sarcoma, (2) the lymphoid celled class, and (3) sarcoid growths. I shall review only so much of the symptomatology as is necessary to a clear comprehension of the condition in point, its histology, its probable relationship to other members of the class and its treatment. Endothelioma is excluded because, arising from lining cells, it is in no sense a connective tissue.

I. Fibroblastic Sarcoma.

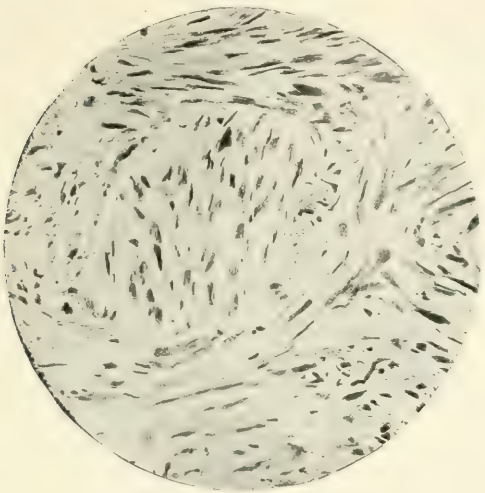
General Considerations.

Symptoms.—Sarcoma is primary, as in the cases reported here, or forms metastases in the skin from internal foci. In either case the neoplasm may be single or multiple, pigmented in varying degrees or perfectly white. It develops either in the corium or in the subcutaneous tissue (when the skin is freely movable over it) and depending on the direction of its growth is seen clinically in the form of flat plates or nodules which are more or less elevated and in rare instances pedunculated. The size varies greatly, from a quarter to several inches in diameter, with perhaps a projection above the surface of two inches or more. In case of injury or from spontaneous necrosis, ulceration occurs, and in course of time the ulcer may take on a fungoid appearance. The course is quite as variable as the clinical picture. Certain spindle-celled and myxomatous growths, the former indistinguishable from others rapidly fatal, are not only indolent, but apparently remain localized for life. There is no clinical sign by which to distinguish the cellular varieties of sarcoma.

Primary disease may appear at the site of an old injury such as a boil (Kaposi) or a scar (fibrosarcoma, Fig. 1), but tissue injury is not a necessary precedent. Hutchinson's melanotic whitlow, of which more is heard in Great Britain than in America, is a sarcoma of the peri-ungual fold. A clinical picture has been several times described in which a parent tumor, it may be a mere pigment spot on the skin, is seen surrounded by a number of daughter growths from which dissemination may be extremely rapid. Sarcoma sometimes pursues an extraordinary course as in the case recently reported by Iwanoff.[1]

The neoplasm was a generalized round-celled one, probably secondary to a breast growth, which followed for two months its usual course, with cachexia, then suddenly developed a condition resembling an acute infection, which terminated fatally in three weeks. At autopsy there were found besides metastases an hypertrophied spleen, fatty degeneration of the liver, kidneys and heart, and a general arterial thrombosis which was neither septic nor neoplastic. No organisms could be demonstrated during life or after death, and the author is in

FIG. 1.



Fibrosarcoma.

doubt whether an acute infection was present or the sarcoma itself produced symptoms of systemic poisoning.

True pigmented sarcoma arises in one of two situations; in the cutis, in spite of contrary statements, or in the choroid. Musser[2] has reported an interesting case of multiple cutaneous metastases from the eye. A primary pigmented tumor may be multiple and its rapidly growing internal deposits, which may far outstrip the parent growth, should not be regarded as the original foci. (The author is not confusing this variety with tumors developing from nævi, which he regards with Unna and his followers as generally of epithelial origin.

It is conceivable that they may, however, arise at times from endothelium. Sight should not be lost of the fact that Lubarsch's doubts as to their carcinomatous character are shared by many others.)

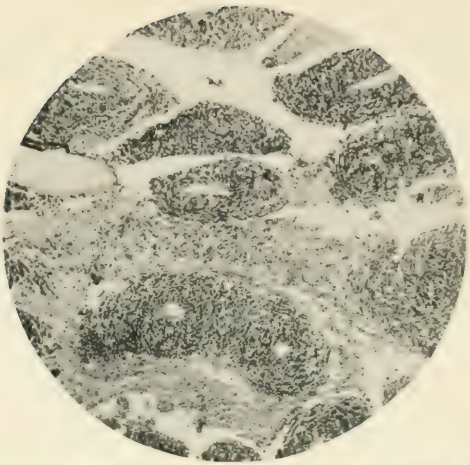
Prognosis depends, aside from a history of indolence and absence of metastasis, on certain histological features to be discussed later, the type of cell, the blood supply, amount of intercellular substance, and presence of encapsulation and pigment. It is my belief that all pigmented sarcomas, when that pigment is autochthonous, are malignant from the beginning. The difficulties in this connection are readily understood since it is common knowledge that sarcoma may change all its characters within a week, but, in general, all rapidly growing neoplasms of this nature, not myxomatous, with little intercellular substance and infiltrating the surrounding tissue, are of the gravest prognosis. Fibrosarcoma and myxosarcoma are sometimes benign; the general rule is that metastasis from them is late. Encapsulation betters the outlook by preventing spread. A sarcoma seems to grow as easily with a rich as with a poor blood supply in certain instances, but ordinarily when it is abundant, with vessel walls not fully formed or absent, growth is rapid. Spontaneous involution is sometimes seen. As to the cell type, the further it departs from the fibroblastic form (barring giant cells) the more malignant it is.

Histology.—Sarcoma is ordinarily divided according to the type of its cells into spindle (fusocellular, Unna), round and myeloid or giant-celled forms. Giant cells may occur in either of the first two types, rarely appear as constituting even the major portion of a tumor, and have no special significance except that when abundant their number points to a bony origin. (See also Buxton.[2]) They are probably developmental anomalies in every instance, even in myeloid tumors, their appearance being due to altered nutrition or simply the presence of foreign bodies. Dubreuilh and Venot[3] have described a sarcomatoid growth in which giant-cell formation was due to the presence of minute fragments of oyster shell, and cite another in which the same appearance was caused by particles of nettle. These discoveries are vastly suggestive. Spindle and round cells are capable of direct transformation, one into the other, so that in reality there is one sarcoma which originates from the fibroblasts either of the adventitia of vessels or of ordinary connective tissue. I will not venture to assert that at the outset all sarcoma cells are spindles, but it seems probable. The type of cell from which a growth takes its name may be perfectly preserved throughout, or may change in the same tumor so that its cells appear spindled in one part and round in another. This anaplasia is illustrated in the following case:

FIG. 2.

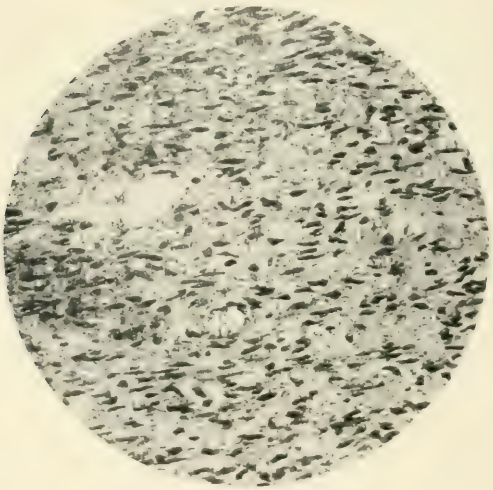
Case I. Perithelial Sarcoma. Metastasis in lymph node, showing perithelial arrangement below and round-celled growth outside capsule. A little lymphoid tissue to left. × 10.

FIG. 5



Case I. Perithelial, spindle-celled sarcoma, vessel lumen showing in cell masses. Lymph node metastasis. x 25.

FIG. 6



Case II. Myxosarcoma. x 250.

Case I. *Perithelial Sarcoma.*—Male, aged forty-seven. Numerous warty tumors scattered over cutaneous surface, two of which showed on examination fibrous tissue only. Two years ago he fell through the floor, and injured the forearm just at the site of a large pigmented nævus. Two months after the accident, axillary nodes became enlarged. Ten months later, a swelling about the size of the palm of the hand gradually made its appearance a little below the axilla. On October 1, 1901, this was removed with the glands, but in one month's time recurred in axilla. The growth was promptly excised, but in the meantime a tumor, the first sign of which was evidenced in July, 1900, in the form of pain, developed in the abdomen.

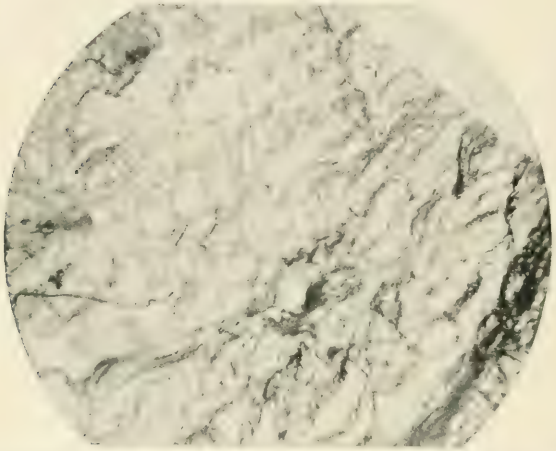
The specimen is from one of the lymph nodes removed at the second operation. The lymphoid tissue is almost entirely replaced by new growth within the capsule, which consists of numerous, probably newly formed, vessels, surrounded by thick sheaths of tumor cells. They are arranged with their long axis generally pointing to the lumen of the vessel and are fibroblastic spindles, with oval, vesicular nuclei and poorly staining protoplasm. The reticulum is everywhere evident under high power. At one point, the capsule has ruptured, and the cells, released from any restraint, have assumed great proliferative activity, with complete change of form. The transition is easily followed from spindles into large round cells, with hyperchromatic nuclei and acidophile protoplasm, little intercellular reticular formation and few vessels. Rapid growth has by pressure obliterated the blood spaces at points, and caused necrosis. There is no trace here of the perithelial arrangement within the node, and very likely, also, of the original growth. This case illustrates another point: that it is not safe to generalize to the extent of saying that all neoplasms arising from nævi are carcinomatous. (Figs. 2 and 3. Case from General Memorial Hospital, for the use of which, as well as all of the accompanying photographs, I am indebted to Dr. B. H. Buxton.)

Reference has just been made to the presence of a fine fibrillar network between the cells. This is the sarcoma *reticulum,* present to a greater or less degree in every sarcoma, and a product of the activity of its cells. Its formation indicates clearly their fibroblastic derivation. The fibrils are best demonstrated by a fresh Van Gieson (picrofuchsin) stain, and under a high power. In a balsam mount the stain disappears from the reticulum after a very few days. W. C. White[4] has shown its presence by Mall's modification of Spalteholtz's[5] method beyond any possibility of doubt, in serial frozen sections, each alternate one being digested in pancreatin, the next stained as usual. The cells disappear after digestion, leaving the intricate network intact, and

showing the fibers at times converging to a central focus, doubtless some particularly active cell (Fig. 4). The reticulum should not be confounded with remnants of preëxistent collagen which sarcoma destroys as it advances, although the new material appears from its staining to be composed of the same substance. The reticulum is *entirely absent between the cells* of both carcinoma and endothelioma, a valuable differential point.

Pigment may be autochthonous; that is, probably elaborated *in loco*, in stable combination with the cell protoplasm, and set free only with

FIG. 4.



Sarcoma Reticulum. Reproduced from White's illustration in "Johns Hopkins Hospital Bulletin" through kindness of its editor.

the death of the host, or hemorrhagic from capillary rupture. In either case it is both extracellular and intracellular, but hemorrhage is usually evidenced by the presence of red corpuscles outside the vessels. Autochthonous pigment is always granular, and Perl's test shows no iron. Blood pigment gives the blue reaction at once. New vessel formation, part of every sarcoma, will be considered elsewhere.

Theory of Growth.—Adami's theory[7] of the "Causation of Cancerous and Other Growths," adapted to the sarcomas, works out even better than in epithelial tumors, it seems to me, although its distin-

guished author has not carried it so far. The function of the fibroblast is that which its name indicates, of course, and its office is never wholly forgotten, even in rapidly growing, round-celled neoplasms. The reticulum is the result.

So long as a cell maintains its physiological function, its "habit of work," it is innocuous, but when that function is laid aside for a regenerative activity, "habit of growth," a tumor is formed, malignant or benign as the surrounding tissue is invaded and destroyed and metastases appear, or not. The fibroblast is not highly specialized, and the assumption of the habit of growth is natural under certain circumstances (process of repair).

When, however, as a result of irritation or other conditions which cannot be explained, the growth begins and spreads by contiguity or through the lymph channels, the activity is no longer physiological. If the habit of work is strong in the fibroblasts, a fibroma or (at least) a fibro- or myxosarcoma results; if not, a round or spindle-celled tumor with little intercellular substance. This theory explains the well-known connection between sarcoma and scar formation, to say nothing of fibroma and its malign relative. I have sections from a sarcoma arising in a cicatrix, in which the gradual transition from normal fibroblast to tumor element is very clear. As a corollary to the idea here presented, it may be said that the greater the amount of work done by the cell, the less energy it has to put into growth, consequently the less malignant the tumor. We should expect to find fibroma, fibrosarcoma and myxosarcoma more or less benign, which is true. They spread slowly, or not at all, and metastasis is rare. Very richly cellular neoplasms are nearly always malignant. Again, the further the sarcomatous cell departs from the fibroblastic type (barring giant cells, which are of no significance except for diagnosis) the more malignant it is, for the reason that it is more incapable of function and more given to regeneration. Unlike carcinoma cells, reversion, histologically complete, to the embryonic connective tissue type, does not entirely preclude function in the shape of fiber formation in sarcoma.

Spindle-celled Sarcoma, primary or secondary, pigmented or not, shows two types of cells, in a pure or mixed state, large and small. The terms are relative, but it is not usual to find extremes in the same growth. It seems hardly possible, as Unna says ("Histopathology"), that spindle cells develop always from the adventitia of vessels. I am inclined to think that such an origin is more liable to produce, ultimately, a round-celled tumor, as in Case I., by anaplasia, but in all such growths the process of transition must be followed, and the retic-

ulum demonstrated before they can be called sarcoma. Perithelial tumors may arise from perivascular lymph spaces, also, and morphologically their cells may be epithelioid and indistinguishable from fibroblastic round cells. An additional point of differentiation lies in the fact that endothelioma is intercellularly avascular. Fordyce[8] and Wolters[9] have reported, with illustration, typical examples of perithelial sarcomas and endotheliomas, the spindle cell type being pure in the former. Winfield's[10] case is a multiple hem-lymph angiosarcoma, new formation of blood and lymph spaces, with proliferation of fibroblasts.

There appears no reason for doubting that connective tissue corpuscles, other than adventitial, are capable of taking on malignant action. The intercellular substance is always more abundant than in other sarcomas, and is in evident relation to the cells, sometimes seen to be continuous with them. It is hardly possible to mistake the new reticulum for collagenous material partly destroyed by the growth. In alveolar and fibrosarcoma, this relationship is particularly striking. At times even a rapidly growing spindle-cell sarcoma, in addition to the network, will elaborate a mucoid substance, which, although the resemblance to myxoma is remote, is called myxosarcoma. The basophilic clear material lies with the fibrils between the cells, and is evidently a secretion, not a degeneration, for the tumor elements are unchanged. The onus of this additional work, while it does not limit the proliferative activity of the cells, keeps their malignancy in bounds. Myxosarcoma is almost benign.

Case II. *Myxosarcoma.*—The patient is a domestic, of English extraction, thirty-one years old. The history is somewhat indefinite, but the area, which shows in the photograph (Fig. 5) atrophic scarring, with sessile nodules here and there, she says, has been present since birth; the large tumor has developed in four months. No mention is made of irritation or injury. The process covered an area, 3x2½ inches on the inside of the thigh. There was no pigmentation and no ulceration, except at the summit of the large mass, where the epidermis had entirely disappeared. The flat surface shows numerous puckered, atrophic spots, where evidently previous growths have undergone regression. The skin is adherent to the scars and sessile nodules. On section the growth is seen to be everywhere limited by the subcutaneous fat. It is white, soft and traversed by numerous vessels. The large mass is cylindrical, an inch and a half high, by one in diameter, with a condensation capsule about its lower edge and sides. There were no metastases.

The cellular form is single throughout, a rather large spindle, with an unusual uniformity in size. The cells bear no particular relation to

the blood vessels, which are numerous, especially near the surface, and fully formed as to lining, so that there is no hemorrhage. Mitoses are fairly common, as would be expected in a tumor of rapid growth. There are no round or giant cells. The limiting capsule shows a beginning change of its fibroblasts into spindle cells. In the central, presumably older portion, evidence of cell division is less common, and in places the cells are separated from each other by spaces filled with a substance which, in sections stained with hematein alone, take a

FIG. 5.

Case II. Myxosarcoma. Gross appearance. Reduced about one quarter.

slightly blue tinge. With picrofuchsin and a high magnification, very fine fibrils stained red are seen traversing the spaces. This is the sarcoma reticulum, and the fact that it takes an acid dye prevents its being mistaken for mucin precipitated by alcohol. In the peripheral portions the cells are more and more closely packed as the capsule is approached and run in bundles without order. The growth begins in the reticular layer and remains limited to it, except where ulceration has occurred. (Fig. 6.)

Blood-vessels in sarcoma, even when of fair size, show a simple

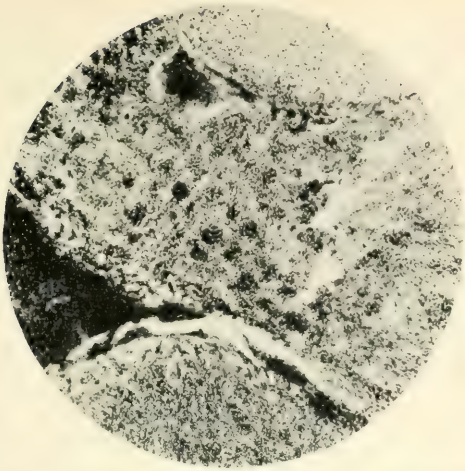
structure, a lining endothelium, with perhaps a little subendothelial connective tissue. When growth is rapid, this lining may wholly disappear, and vascular spaces are walled by spindle cells, a condition which admits of free interstitial hemorrhage and consequent pigmentation. In other tumors (I have never seen this anomaly in any organ except the skin) endothelium proliferates with the fibroblasts, and presents the condition illustrated by the following case:

Case III. *Spindle-celled Sarcoma, with Endothelial Proliferation.*—The tumor was a solitary growth of long standing, in a woman of fifty-six. It was about the size of a hazelnut, unpigmented, with an irregular surface, which led the surgeon who removed it to call it a "papilloma." The epidermis dips, in places, into deep sulci. There is no mention of metastasis. Histologically, the tumor is a spindle-cell sarcoma of the corium of peculiar appearance. With a low power of the microscope there is a regular dotting of the field by dense clusters of cells, which seem at first an artefact, but their regularity and constant appearance precludes this notion. The sarcoma cells generally radiate from these foci, very loosely arranged with wide spaces at the greatest distance from the points referred to. This spacing may have been produced by the hardening process, because it is not present everywhere, and there is no mucinous material as in Case II. Chief interest attaches to the focal points. The cells in them are arranged in whorls, concentrically, as in endothelioma of the dura. They are spindle-shaped from pressure, with nuclei more diffusely stained than the sarcoma cells, and are limited peripherally by a fibrous membrane, which, it may be said, is sometimes difficult to demonstrate (Fig. 7). This appearance is seen also in Kaposi's pigmented sarcoma, but not regularly. These whorls are probably lymph spaces, for the blood-vessels are fairly numerous. The reticulum is present between the sarcoma cells only. (I have shown these sections to the members of the Cornell pathological staff, and to Professor Adami, of Montreal, who agree with my interpretation of the findings as to proliferation of endothelium.)

Mitosis is more common in spindle-celled than in any other form of sarcoma. Rapid growth, so long as the spindle type is maintained, is conducive to their appearance. When division is to occur the cell becomes elliptical, and its protoplasm has a distinctly greater acidophile tendency. Nuclear division takes generally the bipolar form, but at rare intervals it is tripolar. Figs. 8 and 9 illustrate these points well, as to shape and nucleus. Tripolar division may be an early stage of giant-cell formation, but I think it unlikely.

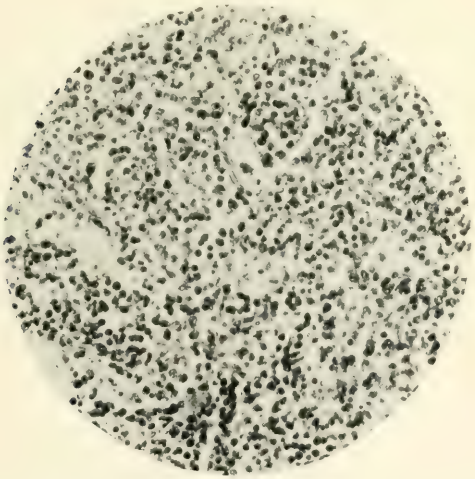
Round-Celled Sarcoma.—The large and small varieties are so different that they cannot, like the spindles, be described together.

FIG. 7.



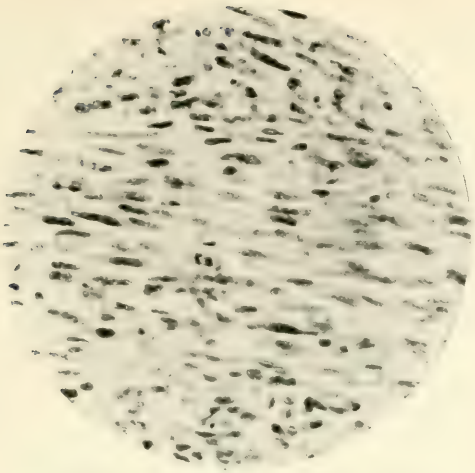
Case III. Spindle-celled sarcoma with endothelial proliferation in whorls. Large vessel to left. x 75.

FIG. 11.



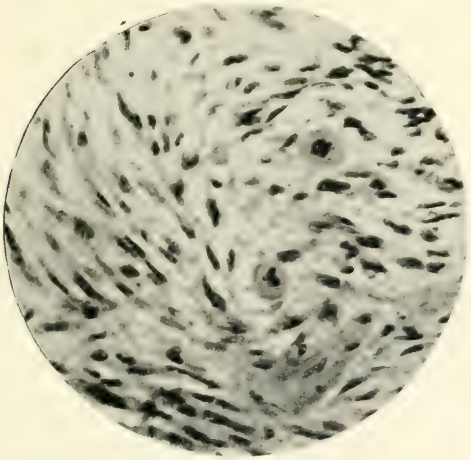
Case IV. Small Round-Celled Sarcoma. Epitheloid Fibroblasts with Reticulum. x 250.

FIG. 8.



Spindle-Celled Sarcoma. Bipolar Mitosis. × 250.

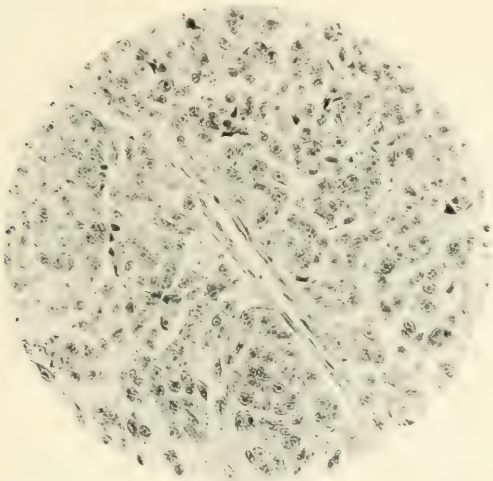
FIG. 9.



Spindle-Celled Sarcoma. Tripolar Mitosis. x 250.

Large, round-celled tumors conform in type to the anaplastic deposits in node and chest wall metastasis of Case I. The cells are derived from fibroblasts and vary in size from that of a lymphocyte to that of the largest giant cell. The nucleus is generally densely stained with a thin or wide ring of acidophile protoplasm. True giant cells are uncommon, the large elements having huge, irregular, chromatin masses, which nearly fill the body. The hyperchromatic nuclei do divide occasionally, but even then do not form true myeloid cells.

FIG. 10.



Large Round-Celled Sarcoma. Epithelioid Fibroblasts. x 250.

Amitotic division is the ordinary event, vascular supply is poor, necrosis common, and the reticulum far more difficult to demonstrate than in spindle-cell sarcoma, because the habit of growth has almost completely replaced the habit of work. There is another growth of large, round cells, which are epithelioid in appearance, nearly uniform in histological character, and almost pure in type, in which the reticulum is easily demonstrated and blood supply abundant. Giant cells are little apt to form in it. (Fig. 10.)

True fibroblastic sarcoma, with small, round cells, has occurred in

my experience only once in the skin, or in any other organ. I am indebted to Professor Ewing for the use of the following case:

Case IV. *Small, Round-Celled Sarcoma.*—The patient was a young woman, who had about eighty of the tumors scattered over her body. The one from which the two sections I have seen was taken was not more than half an inch in diameter, rather deeply imbedded in the skin, and not pigmented. One of the sections I stained with picro-fuchsin, and brought out the reticulum easily, although the preparation was eight or more years old. The tumor is enclosed in a dense condensation capsule, formed by pushing aside the connective tissue, and lies wholly in the cutis. It is tolerably well supplied by blood vessels whose walls seem fully formed as to lining endothelium. The neoplastic cells are small and uniform in size, about the diameter of a polynuclear leucocyte. Except for size, they are like the second variety of large, round cells, epithelioid, with a granular acidophile protoplasm, and a round, central, vesicular nucleus. Mitosis is uncommon, perhaps because growth is restrained by the tough capsule. I cannot make out that the cells have any particular relationship with the vessels; they are too closely packed. (Fig. 11.)

Giant-celled sarcoma occurs in the skin by extension from bone marrow or periosteum, in the former case through the osseous covering. These sarcomas always show giant cells, no matter whether they grow without hindrance or not, in which they differ from the accidental formation in other sarcomas. The tumor from the marrow should be called strictly myeloid, myelogenous, or medullary sarcoma, the other periosteal, for the two are not identical, and the latter is more benign. Neither forms metastasis, but the growth may be multiple at the start. Both contain numerous vessels, much intercellular substance, with a structure like that of epulis, a mass of small, round spindle, true myeloid giant cells, and cells with hyperchromatic nuclei, which have been called myeloplaxes. The last term is probably a misnomer: the cells are probably formed as in perithelioma, by a simultaneous overgrowth of chromation and protoplasm. The nuclear outline is often fluted. Delbanco[11] has reported a periosteal tumor which existed on the finger for twenty-two years. Heurteux[12] and Venot[13] have collected a series of such cases. I should bar them all from consideration in dermatological literature, because they involve the skin, like a deep abscess, only by contiguity. I have seen one case in which the skin was reddened, and attached to a tiny periosteal tumor.

Treatment.—There is some hesitancy even in recommending thorough and early ablation. Putting a knife to a quiescent sarcoma is often

touching a match to a gasoline can. It shortens the patient's life. A solitary growth of small size, or encapsulated, or of the fibrous or myxomatous types may be removed, with the happiest result. It is almost useless to tamper with disseminated tumors, except to relieve symptoms; their cells surely lurk in intervening lymphatics. However, the off chance must be taken in the beginning. Inoperable cases may be tried with Coley's toxins, and offer a fair prospect of success in selected instances, from the fact that injection may be made directly into the tumor mass. There is no doubt that the toxins have cured, and have held malignancy in check, but from what I can gather at first hand nonpigmented, spindle celled sarcoma offers the best if not the only prospect of success.

As to the therapeutic value of arsenic, divergence of opinion, notable even in dermatology, exists. As a last resort, it may be given always by injection into the subcutaneous tissue or into the tumor, every second day. The dosage is 7 cgm. of cacodylate of sodium, 2 cgm. of sodium arsenite, and 10 drops of an equal part mixture of water and Fowler's solution, increased gradually to toleration. Arsenic does exert an extraordinary influence on many fibrous neoplasms of the skin, and one would expect to find it useful in the sarcomas which nearly approach them.

II. Lymphoid-Celled Group.

In the present scheme, this subdivision includes leukemia, pseudoleukemia, and malignant lymphoma. The last is also variously styled lymphosarcoma, and small, round-celled sarcoma, with little justification except that of usage. It is not possible to enter upon a discussion of the relationship of these disorders, but in their early stages, before lymphocytosis appears or in intervals of remission, at least, lymphatic leukemia and Hodgkin's disease cannot be differentiated. The same absence of dividing line exists between pseudoleukemia and lymphoma. Nékám[14] states only those cases can be accepted as leukemic which occur in the course of leukemia; a correct restriction, but one which does not forbid their admission to this group. When leucocytosis reaches its height, the difficulty, so far as leukemia is concerned, is removed. There can be no certainty as to a differentiation between Hodgkin's and lymphomatous deposits, but it is probable that when the lymphoid cells proliferate in the skin, and especially wher true giant cells are formed (providing syphilis and tuberculosis are excluded), we are dealing with a true neoplastic formation. Pinckus'[15] discovery, that in leukemia the skin growths arise from preëxistent lymphatic tissue, not from lymphocytes, is unworthy of consideration, because no normal

lymphoid tissue exists. He describes giant cells as part of the process in his case, a finding which throws great doubt upon his diagnosis. Large acidophile cells are seen in certain lymphomas, which are probably only altered endothelium.

So far as the skin is concerned there is no question that all three processes produce true tumors. In leukemia and pseudoleukemia the growths are derived, according to both Oertel[15] and Nékám, by emigration of leucocytes, increased and renewed from the same source; they are metastatic deposits in lymphoma. A large number of cases has lately been eliminated from this category by the discovery of the tubercle bacillus in section and by inoculation of the nodes, no typically tubercular changes being present.

All sorts of anomalous cases have been noted, particularly by Kaposi, in which it was impossible to determine the character of the disorder except that its cells were lymphoid. These indeterminate cases which, according to him, "may shade into leukemia, pseudoleukemia, or mycosis fungoides," are described as follows: They occur in anemic females whose skin on face, trunk, and extremities becomes—except for small, healthy islets—smooth, pale, red, and glossy. It is thickened sufficiently to cause an appearance of enlargement of the parts. The consistency is doughy, with firmer masses in deeper parts. The skin is hyperesthetic, so much so as to prevent scratching, although itching is intense. All of the patients have died, usually from marasmus, "preceded by development of lymphatic abscesses, fungoid tumors, adenopathy, or true leukemia."

The lymph nodes in the three affections show the same microscopical picture, an increase in the small round-cells, which pack the lymph spaces. Hodgkin's disease may be only a hyperplasia and lymphoma a tumor, but it is a wise man who can say where one process ends and the other begins. Lymphoma is usually seen first in one node, pseudoleukemia in a group, but this rule may be reversed. Both are accompanied by grave anemia. Lymphocytosis, if it is ever present, is not persistent, as in leukemia. Lymphoma occurs in the skin only in metastasis, a single or multiple white mass, whose nature must be determined by the microscope.

Leukemia and Pseudoleukemia.—It has been well established that both a diffuse and circumscribed tumor formation may occur in the skin in each of these affections. The diffuse lesion is lymphodermia perniciosa of Kaposi, and begins in the form of a partly diffuse, partly localized, eczematoid dermatitis, scaling, moist, and intensely pruriginous. Gradually the skin becomes thickened and nodules appear in it, here and there, which may ulcerate. In Kaposi's classical case there

was "absolute increase of white corpuscles," and at autopsy nodules were found in the pleura and lungs, the spleen was four times its normal size, and the bone marrow was grayish from "excessive leucocytosis."

In other cases nodules appear located in the deeper tissues, white or bluish, with or without pigmentation and no involvement of the intervening skin. In the example reported by Neuberger there were two symmetrical tumors on the cheeks without any other lesion. The growths are rarely attached to the skin, but at times the whole cutis is invaded, and ulceration takes place. Fungoid masses may develop on the ulcer, as in the transitional cases. Arning reported one instance, in which pseudoleukemic tumors were found in the mouth and imbedded in the muscles; Hochsinger and Schiff, a remarkable case in a child of eleven months, in whom the tumors, varying from a pinhead to a hazelnut, were disseminated over the whole body. The neoplasms have the same clinical appearance in both diseases.

In the later stages of Hodgkin's disease there may appear a condition called pseudoleukemic prurigo, one case of which I have seen. It is evidenced by tiny, pale papules, which itch furiously, and becoming excoriated by the nails, immediately are capped by a punctate blood crust. The skin is deeply pigmented, either from the cachexia or as a result of the incessant scratching. Tumors are not necessarily present. The phenomenon is not surprising, since Kreibich has found infiltration in macroscopically unchanged skin.

Histologically, in their metastases, if we may use such a word, leukemia and pseudoleukemia are identical. The neoplasms can be differentiated only by the blood count, when it is characteristic, and by concomitant symptoms. They begin always in the deeper tissues, commonly at the junction of reticular layer and subcutaneous tissue, in the form of perivascular sheaths of round-cells. These cells have the usual appearance of lymphocytes, a narrow ring of protoplasm, which takes basic dyes (methylene blue), surrounding a rounded nucleus usually diffusely stained. Other forms are present in smaller number in lymphemia, the large mononuclear and transitional cells. Myelogenous leukemia or myelemia, as it is coming to be called, while transition exists between it and lymphemia, is not likely to be mistaken for the latter or for Hodgkin's disease when myelocytes can be found in smears from the peripheral blood or in the tumor tissue. They are difficult to demonstrate in section. Oertel's,[16] Nékám's and Kreibich's[17] cases seem to be all of the myelogenous variety. Polynuclear leucocytes are sometimes present.

The cells, myelocytes, or lymphocytes have no relation like that of fibroblasts to the intercellular collagen. They do not arise from it, even

in lymphoma, and their growth merely pushes it aside and does not destroy it as sarcoma does. In the nodes and metastases of the whole class it is not unusual to find here and there a fine thread which stains with orcein, and is an elastic remnant, caught and held as the growth pushes other tissue out of its way. Blood-vessels are few and penetrate the tumor only in septa between its cellular masses, not between the cells themselves.

Treatment.—Removal is, of course, useless. Coley's toxins are absolutely ineffective, and arsenic is the only avenue of escape, generally a blind alley. In Arning's case of pseudoleukemia a marked improvement was noted. Tomon's recovered entirely under the use of the drug, and Zeisler stated quite positively, in a recent address, that it affords a prospect of cure, so that its aid should always be invoked. It is given as in sarcoma.

III. Sarcoid Growths.

"Sarcoid" is a curious, hybrid term, which has, however, become intelligible by use. As the disorders occur, except in the rarest instances, in the skin alone, they have apparently escaped the notice of the general pathologist. Gaylord tells me he has seen a growth very like benign sarcoid in the lower animals.

The members of the group have been bones of contention since Alibert first described mycosis, or, as it is now called, granuloma fungoides, early in the nineteenth century. The class has close relationship, on one hand, with sarcoma, and, on the other, with granuloma, consequently with productive inflammation. No infective agent has been demonstrated by culture or inoculation in the causation of any of the group, a gap which may be filled when methods are less faulty.

The diseases to be considered are: (1) Granuloma fungoides; (2) multiple idiopathic pigmented sarcoma; (3) sarcomatosis cutis, and (4) multiple benign sarcoid. There is no need to review the familiar clinical features of the first two. It will be noted that there is singular unanimity of opinion as to their nature among later histopathologists. Metastasis in internal organs has been reported once in the first and in one doubtful instance in the second. Except granuloma fungoides, the class is, in most instances amenable to treatment by arsenic.

Granuloma Fungoides.—Not all of the investigators with whose views I am acquainted are agreed that the disease is a granuloma, whose cause has escaped detection, not a sarcoma, but the opinion that such is its nature, backed by Unna, Kölbner, Crocker, Philippson and Leredde, Hochsinger and Schiff, and Payne, is not to be controverted,

except on findings which will overturn completely present conceptions in the cytological field. Vidal and Paltauf[17] have made an effort to identify the growth with lymphodermia, on grounds which are entirely insufficient. As my investigations on the case autopsied by Elliot some years ago agree entirely with Galloway's,[18] expressed in his admirable paper, I shall quote largely from that source.

The skin shows in all stages of the disease the same character of cell infiltration. It has even been demonstrated in areas in which no change is clinically apparent. The process begins about the vessels of the corium, as well as round the sweat-gland and hair-follicle plexuses, in the shape of an infiltration of lymphocytes, plasma and mast cells, the last in no great abundance, and a proliferation of connective-tissue cells. The proliferation furnishes the bulk of all the foci. Its cells are large, round, oval or fusiform, with a granular protoplasm, and large, vesicular nuclei, and a smaller variety with the same characteristics. A reticulum is found between these cells at first like that of sarcoma, but later disappears almost entirely. In early lesions (in any stage) these connective tissue cells divide by mitosis, but, when growth becomes rapid in the tumor period, division is amitotic and extremely rapid. A few giant-cells are to be found. The epidermis is affected secondarily, and shows eczematous changes, acanthosis, intercellular edema, and parakeratosis. In later stages, simultaneously with, perhaps because of, rapid proliferation and consequent vessel obliteration, the connective cells break down. Accompanying their necrosis there is change in the collagen and elastin, which finally disintegrate. The epidermis is encroached upon, is flattened, and finally disappears, leaving an ulcerated surface. Upon this the "tomato" tumors appear. Various organisms have been found, but they were properly regarded as contaminations, or, like McVail's[19] "white bacillus," have failed of confirmation.

Malherbe and Monnier[20] have reported a case in which there were metastases in the axillary, inguinal, bronchial, and mesenteric nodes, in the breasts, lungs, pleura, kidneys, pancreas, uterus, ovaries, and heart. The case cannot be regarded without suspicion, as in addition to the extraordinary dissemination, the authors describe a "marked leucocytosis," and class it with lymphadenoma.

Multiple, Idiopathic, Pigmented Sarcoma.—Histological findings are practically uniform throughout the great number of reports now available. The disease begins simultaneously at several points on the extremities, in the lower portion of the cutis and subcutaneous tissue. The cells are spindle shaped and run in bundles longitudinally, transversely and obliquely, their cross sections giving the appearance of

round cells. Mitosis is quite common and an intercellular reticulum is readily demonstrable. The characteristic feature of the growth is the great number of new blood-vessels. Their endothelial lining is incomplete in places and interstitial hemorrhage is frequent. Red blood-corpuscles and pigment granules are always to be found between the spindle-cells. The endothelium of the new vessels takes on a proliferative action in the older portions, varying in degree from a mere swelling of the flat cells to a condition in which the lumen is completely blocked by concentric layers, the cells elliptical from pressure. Involution is brought about by death of the tumor cells, reapsorption of their detritus and the pigment and organization with the formation of fibrous tissue about the new vessels. Kaposi[21] has reported a case in which there were metastases in the internal organs, but as the cells are described as round it is possible that the tumor was not identical with the one under discussion. Lymph nodes are not involved. Fordyce[22] thinks that on account of the characteristic appearance in simultaneous multiple growths on the extremities an infective agent is at work. Wende[23] agrees with him. Sellei[25] states that the process conforms closely to Török's definition of granuloma and ventures to change the name to indicate this nature. Other authors are content to let it pass as a sarcoma. Pringle's[24] finding of bacilli in the capillaries and sweat glands in two cases lacks confirmation.

Sarcomatosis Cutis.—This disease is called by Kaposi[26] "the third type of sarcomatosis cutis," the others being mycosis fungoides and lymphodermia. It is seen in persons of middle age, in the form of numerous, pale nodules, beginning in the reticular layer or subcutaneous tissue. The borders of the tumors are not sharp, but shade gradually into the surrounding skin. The earliest are only to be felt by careful palpation. As the nodules increase in size they take on a reddish tint, with a smooth, shining surface. Coalescence produces flat, raised plaques, the size of the hand, or lobulated masses not unlike the tomato growths of granuloma fungoides. Differing from multiple, idiopathic, pigmented sarcoma, the larger tumors ulcerate, and present a punched-out opening leading to a larger cavity beneath. There is no involvement of lymph nodes, and no change in the blood, except anemia. It is even stated that cachexia does not occur.

The best description of the histology is given by Fendt.[27] He says the growth is made up of large, round cells, which are encapsulated in some nodules, and infiltrate the cutis in others. The elastic fibers remain unchanged, but the collagenous disappear in the infiltrated portions. The cells take stains well, both nuclear and protoplasmic, at the periphery of the nodules, but at the center undergo retrograde metamor-

phosis and lose their staining power. The nuclei are fragmented. Large vacuoles appear in the protoplasm during regression. When the necrotic process reaches the surface, ulceration occurs, and if the detritus is emptied out a cavity with a small opening is formed. The cells seem to be epithelioid, and are of connective origin. (There are no illustrations.) Blood-vessels are fairly numerous. I can find no mention of a reticulum which must be present. Fendt makes an unqualified assertion that the process is granulomatous.

Multiple, Benign Sarcoid.—A description of the disease cannot be given better than in the summary appended to Boeck's original article[25], both as to clinical features and histology. He had then met with only three cases, unless the disease called by Hutchinson "Mortimer's Malady" can be included. The two differ in some particulars. Professor Boeck reports a fourth case in the Kaposi Festschrift, 1901.

"Clinically, we find in a middle-aged, pale, thin man, groups of lymph nodes much swollen, and on examination a slight augmentation of the number of white corpuscles. At the same time there exists a widespread, somewhat symmetrical eruption, firm nodules of varying size, on head and extensor surfaces of trunk and extremities. They range in size from a hemp-seed to a bean, and the larger have irregular contours. They involve the whole skin, and are movable with it. Only on the scalp is the infiltration not palpable. Here only yellowish outlines are seen. The color of the early nodules is bright red, becoming darker, and, finally, yellowish or brown. Slight scaling occurs on older lesions. They show a tendency to peripheral spreading and central depression. On the face, they have a peculiar appearance, with blue center and yellow border, a feature present in all the cases I have seen. The nodules disappear finally, leaving, as a rule, a loss of substance in the skin, which may be white on the face, yellow on the back, and darker at the periphery on the legs. Exudation, ulceration never take place. A papular eruption grouped like lichen planus was seen on the inside of the thigh. A tendency to develop at the site of old injury should be remembered. The symmetry is not such as is found in affections whose localization is evidently determined by central nerve influence. The disease seems to be benign, and disappears under arsenic, or perhaps spontaneously."

The histology is unique. "The areas of new growth might be described as perivascular sarcomatoid tissue, built up by excessively rapid proliferation of epithelioid, connective-tissue cells in the perivascular lymph spaces, with little addition of other varieties. The tumor soon begins to degenerate, and the tissue is rarefied, showing a network of branched connective-tissue cells. It should be remembered that true

giant cells of sarcomatous type were found, though rarely. Compared with other new growths of the skin this must be said histologically to possess affinity to sarcoma and also to the cases of pseudoleukemia cutis described by Arning and others." I confess I cannot follow the last analogy. The name indicates the place for the neoplasm, though no organism was found.

Conclusions.

The processes of elimination and classification are going on in sarcoma cutis, as in other groups, such as eczema and the bullous affections, and is unquestionably a step in the right direction, whether my deductions are accepted or not.

1. The group may be divided into three classes: Fibroblastic sarcoma, the lymphoid-celled class, and sarcoid growths.

2. The first class comprises spindle and round-celled tumors. True giant celled sarcoma is not primary in the skin. The origin is the same for all types, from the fibroblast. Between the cells there is present invariably a delicate reticulum, probably composed of collagen, which does not occur in epithelial neoplasms. The tumors are all vascular.

3. The lymphoid-celled class should have a place to itself. In lymphatic leukemia, Hodgkins' disease and lymphoma, the cells are all of one type, and the cases show transition states from one to the other. There is no intercellular substance present as a product of the new cells, there are no vessels ramifying between them, and the preexistent tissue does not melt away before their advance. So far as the skin is concerned these disorders give rise to true tumor formation, which may be asserted also of myelogenous leukemia. This class is not related histogenetically to either of the other two.

4. The sarcoid tumors are undoubtedly all fibroblastic in origin, but two of them are probably granulomas, whose organism has not been discovered, no reproach at present. They are granuloma fungoides and sarcomatosis; with them should be classed Boeck's sarkoid as a granuloma, certainly not with lymphodermia. Modern histologists regard idiopathic sarcoma as having the same character, but it closely approaches sarcoma on one side and endothelioma on the other. The reticulum is present here, as in sarcoma and productive inflammations, but these neoplasms do not metastasize, and they are curable by arsenic, except granuloma fungoides. Perhaps the spindle-celled sarcoma, which disappears under the same treatment, is a near relative to Kaposi's sarcoma, so that a transition may be traced by easy and well known steps from the most malignant of neoplasms to benign tumors, hardly distinguishable from granuloma, a class of infective, generally

productive neoplasms, as lymphoid tumors shade from lymphoma through lymphemia to inflammatory hyperplasia. It will be interesting to see whether an infectious agent is ever discovered for fibroblastic sarcoma, or whether a sharp line is to be drawn through the sarcoid group, between it and granuloma.

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FLAGELLA STAINING WITH NIGHT BLUE.

LETCHWORTH SMITH, M.D.

(*Pathological Laboratory, Cornell University Medical College, June, 1901.*)

The staining of the flagella of various bacteria according to the directions given in most handbooks is not entirely satisfactory. Most procedures require the use of two solutions, — a mordant and a stain, — and this makes it difficult to locate the error in case of failure. While the method given below may not, in experienced hands, prove better than that of van Ermengem or Pitfield-Muir, its simplicity and the ease with which moderate success may be obtained seem to justify bringing it to the attention of those interested — more especially those who would be glad to find a process suitable for the use of students with limited time at their disposal.

The principal details were obtained from Dr. George H. Newman, Medical Officer of Health for Finsbury, London, and may be found in the second edition of his "Bacteria" (John Murray, London).

Materials. — Cultures should be fresh — for B. typhosus, B. coli communis, and the like, sixteen to twenty hours' growth on agar.

Slides or cover glasses may be used. In either case the surface must be very clean and free from scratches. For cleaning, nitric acid followed by absolute alcohol is usually sufficient, or they may be boiled in strong soda solution, but a final washing with soap and hot water, and drying with a fresh soft cloth, often leads to better results. Any inequality of the surface increases the precipitate and prevents it from being washed away. The slides or covers should be carefully selected, as even new material is often badly scratched.

Flagella stain:

Tannic acid, 1 gm.

Potassium alum, 1 gm.

Distilled water, 40 c.c.

Dissolve by a few minutes' shaking, or leave in the incubator over night.

Dissolve night blue[1] (dry) 0.5 gm. in 95 per cent, or absol. alcohol 20 c.c. Mix these two solutions thoroughly, and remove the heavy precipitate by filtration. The stain will not act satisfactorily when more than a few days old, and unless used immediately must be filtered again directly before use.

Process. — 1. An emulsion — to very faint cloudiness — is made by carefully adding one or two loopfuls of the fresh culture to a small quantity of distilled water in a salt-cellar. Avoid scraping the surface of the agar, as every particle of extraneous material will stain and detract from the final picture. The emulsion should be used as soon as possible after making.

2. Transfer a loopful of emulsion to clean, dry slide or coverslip, spread very gently, and allow to dry in the air.

3. Special fixation is unnecessary, though it seems better to expose to a mild degree of heat for a few seconds. Hold in the fingers and *dry* thoroughly rather than *heat*.

4. The flagella stain is filtered on to the dry film and left in contact for five to ten minutes.

5. Wash carefully but thoroughly in water.

6. Apply a saturated *aqueous* solution of gentian violet for about two minutes to stain the bodies of the bacilli.

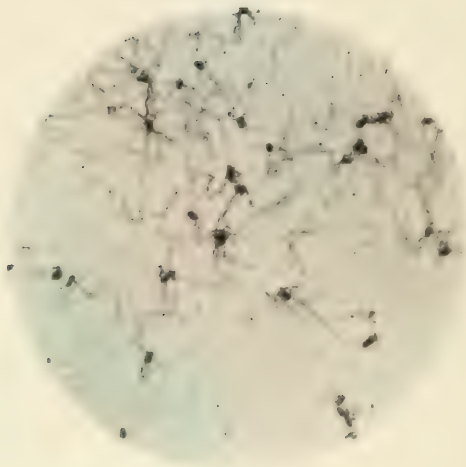
7. Wash in water, dry with smooth blotting paper, and mount in balsam.

If one wishes to obtain a background as free as possible from precipitate, as for photographic purposes, it is better to apply the night-blue stain to the slide inverted as follows:

In a Petri dish are placed a small quantity of the stain, and a match stick to support the end of the slide. When exposed to the air a thin film forms on the surface of the stain. This should be pushed aside with the end of the slide, which is applied to the stain face down.

It is generally true that the conditions which bring about a marked staining of flagella, cause at the same time a heavy precipitation throughout the whole field, but following

[1] Bottle now in use is marked "James Strang & Son, Glasgow & Manchester."



LEICHWORTH SMITH. FLAGELLA STAIN.

the precautions relative to filtrations, clean and smooth glass, inversion of the preparation (when necessary), and keeping the emulsion free from particles of culture medium, the method will be found simple and satisfactory. The photograph is added through the kindness of Dr. B. H. Buxton.

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9. REPORT OF A CASE OF IDIOPATHIC MULTIPLE SARCOMA OF THE SKIN - - - - By HENRY H. KOEHLER and JAS. C. JOHNSTON
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10. A CASE OF BANTI'S DISEASE, WITH DIFFUSE PRODUCTIVE NEPHRITIS - - - - By CYRUS W. FIELD
From Amer. Jour. of Med. Sci., March, 1903.

CONTRIBUTION TO THE PATHOLOGICAL ANATOMY OF MALARIAL FEVER.

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INTRODUCTION.

As with many other infectious diseases, the pathological anatomy of malarial fever had been thoroughly studied long before the discovery of the parasitic cause of the disease. Of the early studies it may be said, from the standpoint of our present knowledge, that they had nearly exhausted the subject of the more evident pathological changes in the viscera, and were essential, indeed, to the final discovery of the parasite, but that in the absence of a knowledge of *Plasmodium malariæ* their numerous misconceptions greatly detract from their value for present purposes. Of current writers,

Laveran is probably most familiar with the value of these studies, and in the last edition of his work properly honors them with abundant reference. Since the discovery of the parasite the entire field has been extensively reviewed, principally in Italy and France, and to some extent in America, Germany and England.

Nevertheless the records of close microscopic study of the lesions of ordinary malarial infection are by no means excessively numerous; much yet remains to be learned, possibly from a microscopic study of the visceral lesions, concerning the parasitology of the disease, while its numerous irregular forms still offer one of the most inviting fields for pathological research. An increased value still attaches to such records from the fact that malaria is seldom fatal in localities where a full clinical history can be supplemented by a complete microscopic examination of the viscera.

Considerations of this nature seem to justify the detailed character of the present contribution, which consists of the available clinical histories and the records of the gross and microscopic changes in the viscera of several cases of malaria, together with a discussion of any important features that the cases may present and their bearing on previous studies.

Most of the material was collected at Camp Wikoff, Montauk Point, Long Island, and the remainder was secured at Roosevelt Hospital or elsewhere in New York City. For the clinical records and notes of some autopsies of the Montauk cases, I am greatly indebted to Dr. Delafield of New York, and to Drs. Cotton, Allen, and Mosher of Boston. Case I, at Roosevelt Hospital, was under the care of Dr. W. H. Thomson, who kindly placed the clinical records at my disposal. Dr. Eugene Hodenpyl and Dr. John H. Larkin have each kindly contributed material and autopsy notes of one case.

PART I.

REPORTS OF CASES.

Case I.— *Estivo-Autumnal Malaria. Sub-Acute Course. Blood Containing only Crescents for two weeks before death. Prolonged Coma. Absence of Parasites in the Brain. Peculiar Deposits of Pigment in Renal Epithelium.*

F. H., 64 years. No important previous illness. Spent some time in Long Island City, N. Y., shortly before illness, which developed in New York City.

September 25, had a chill followed by fever and sweat. Chills recurred every other day till October 2-3, when they became irregular and less marked. Admitted to Roosevelt Hospital, service of Dr. W. H. Thomson, Oct. 6, 1896. Diagnosis, malaria. Treatment, quinine and ginger, aa. grs. XXV-XL. daily. Arsenic later. Fowler's solution, gt. XV. daily.

The patient seemed to improve slightly, the temperature falling gradually, reaching 99° F. on Oct. 10, and remaining at about that point until Oct. 20. There was from the first marked insomnia and tendency toward mild delirium at night, partly controlled by sedatives, until Oct. 11, when the delirium increased, and periods of mild coma supervened. About Oct. 16, the coma deepened and became continuous till death. There were no evidences of uræmia, and the coma was clearly of the malarial type. There was one slight paroxysm of fever on Oct. 20-21 (101.4°), and on the 23rd the temperature began to rise steadily, reaching 108° on the 25th, just before death.

The *urine* was passed in considerable quantity, of acid reaction, 1020 sp. g., and in the last two weeks of the disease contained a trace of albumin and a few hyaline and granular casts. No mention was made of pigment in the urine.

I had no opportunity to examine the *blood* until Oct. 12, when it was found to contain an enormous number of spheroidal, ovoidal, and young crescentic bodies, as many as ten appearing in one field of the immersion lens. Prolonged and repeated search (4-5 hours) failed to show any rings. There were the changes of secondary anæmia with marked loss of Hb. and moderate variation in the size of the red cells. The leucocytes were slightly reduced in numbers. Mononuclears 35%; polynuclears 60%; eosins 5%. A few richly pigmented mononuclear cells were seen. Oct. 13. The parasites were as numerous as before but there were now some elongated and apparently full grown crescents, while the spheroidal bodies were less numerous. No rings seen. Oct. 15. The adult crescents now outnumbered the smaller forms, which were however still rather abundant. No rings could be found. The anæmia appeared rather more pronounced. There was no leucocytosis, but the eosins were still increased. Oct. 21. The numbers of parasites were still large and the forms were about equally divided among elliptical bodies and adult crescents. Oct. 25. Eight hours before death the blood was found to contain very few parasites. In the course of this search 8-10 young crescents and spheroidal bodies were encountered, but no rings. There was a moderate polynuclear leucocytosis.

Autopsy.—12 hours after death. The anæmia appeared slight. No œdema. No jaundice. *Lungs*, moderately congested and œdematous. *Spleen*, slightly enlarged, very soft, of characteristic slate color. *Liver*, slightly enlarged, of characteristic slate color. *Stomach and intestine*, negative. *Serous membranes*, slightly discolored in places. *Kidneys*, size about normal; capsules adherent in places, elsewhere surface is smooth; cortex slightly irregular; markings distorted in places. The cortex is light red in color, the medulla and papillæ very dark red, or rusty. The *marrow* of ribs and vertebræ is hyperæmic, and of slight chocolate tinge. *Brain*, moderately œdematous; not discolored; shows no petechiæ; the basal vessels appear normal.

Microscopic Examination.—Liver.—There is a very extensive deposit of pigment, in the form of discrete and conglomerate grains and masses, in the endothelial cells, macrophages, and occasionally in the liver cells. Much of this pigment is very compact and dark, and is evidently of rather old formation. The numbers of pigmented spheroidal or ovoid bodies are considerable, and many of these lie free in the capillaries, but all stages of the ingestion and destruction of parasites by phagocytic cells can be followed. Some peculiar bodies, possibly derived from malarial organisms, are to be seen. One of those rather frequently seen is spheroidal, a little larger than a red cell, with a thick shiny hyaline outer border, and showing a central mass of yellowish pigment granules. In some of the larger vessels there are several spheroidal bodies twice as large as a red cell, hyaline and faintly bluish stained throughout, and exhibiting a moderate number of central pigment granules. A good many small pigmented parasites were seen.

Spleen.—The deposit of pigment is extreme. Much of this is old pigment in compact masses within phagocytes, but there is a considerable number of free pigmented parasites, and all stages of their ingestion and destruction can be seen. Neither rings nor rosettes are seen. There is the usual cellular hyperplasia of the pulp cords.

Marrow.—The marrow of the ribs and vertebræ contains a moderately rich deposit of pigment which is usually limited to the phagocytes. Very little pigment is seen in the vessels, and very few parasites were identified either in smears or sections. The eosinophile cells and giant cells are increased. The nucleated red cells are abundant and some are slightly increased in size.

Kidney.—There is a moderate chronic diffuse nephritis with growth of new connective tissue in small wedge-shaped masses in the cortex. The glomeruli are apparently normal. The tubules show swelling of the lining cells, and in a few places the cells of the convoluted tubules are necrotic. They all contain much granular yellowish pigment giving the reaction of hæmosiderin. Very few parasites could be identified. The deposit of pigment, however, is very abundant and of peculiar distribution. The glomeruli contain more than the usual number of pigmented cells of the usual character. The larger vessels of the cortex are sometimes injected with blood in which are considerable deposits of brown granular or crystalline pigment. In the neighborhood of these vessels there are often many clumps of similar pigment and these are sometimes found in the lining cells of the convoluted tubules which are elsewhere entirely free from brown pigment. The limitation of this pigment in the cortex to the vicinity of vessels, strongly indicates that the crystalline deposits have resulted from the diffusion of dissolved Hb. or of escaped red cells.

Throughout Henle's loops, and especially in the ascending arms and in the collecting and discharging tubules the number of clumps of pigment is enormous. The vessels here, while injected with blood, are nearly free from pigment, which lies exclusively *in the lining epithelial cells of* the tubules, some cells containing 40-50, or more, clumps in a single section (Plate XI, Fig. 9). These cells, moreover, fail to show marked evidences of granular or fatty degeneration, or of fragmentation, but their protoplasm is uniformly finely granular, their edges are unbroken, and their nu-

clei unchanged. On close inspection these pigment clumps are found to be composed usually of small blunt-pointed crystals, sometimes of granules, of dark brown color, arranged usually in a rather compact circle or sphere. Some of the pigment is more diffuse. Occasionally a clear space surrounds the pigment clump, but the great majority of the pigment circles lie bare in the cell.

Brain.—Throughout the medulla, cerebrum, and cerebellum, the vessels are nearly free from pigment and parasites. In some sections from the frontal cortex there are a few pigmented endothelial cells and an occasional pigmented parasite, but most capillaries, though considerably injected, are free from all traces of parasites or their derivatives. In many of the pericellular lymph spaces throughout the cortex there were peculiar structures, the nature of which I have been unable to determine. These bodies consisted mostly of elongated fibrils or rods with tapering ends, about 0.5 to 1 μ in thickness, and 5-15 μ in length. They were sometimes single, more often multiple, and arranged in rosettes, or spirals, or in concentric layers, or irregularly clumped. They stained densely with methylene blue, faintly with hæmatoxylin. Similar deposits were found in other cases of malaria, and in one case of tuberculous meningitis. They may for the present be classed with the artifacts of nervous tissue.

Epicritical.—In the above somewhat anomalous case there are several features of interest. The development of a case of fatal malaria in a patient who for twenty-five years had not been away from the vicinity of New York City is unusual. While autopsies on cases of malaria are not extremely rare in this locality, they are usually cases which were infected in southern latitudes.

While quinine in moderately large doses, with arsenic, controlled the active sporulation of the parasite and reduced the temperature, the treatment failed as usual to have any effect upon the crescentic forms, which persisted in enormous numbers until, rather suddenly in the last few days of the disease, they disappeared almost entirely, although the patient died in hyperpyrexia. This pyrexia is no indication of a failure of quinine to control the infection, as none of the young forms were seen after Oct. 12, and the terminal fever must be referred to other causes.

The prolonged delirium and coma are the chief clinical features of the case. There seemed little ground for doubting that the mental condition was referable to the malarial infection, because the coma was established before the urine contained casts and albumin; the changes in the urine were never marked; there were none of the usual concomitant signs of chronic uræmia, such as œdema, muscular twitchings, etc. The general condition of the patient was typically that of malaria; microscopic evidences of extreme malarial infection were

found in the blood, liver, spleen, marrow, and kidneys, while the evidences of nephritis were very much less marked than those usually found in cases dying in chronic uræmia. Neither can the coma be referred to the presence of organisms in the cerebral vessels, as none were found there, and it becomes necessary to regard the cerebral symptoms as dependent upon other conditions, probably toxic, associated with the severe malarial infection. This conclusion is in accord with the evidence furnished by other cases of the present series, which fails to support the view that malarial coma is always dependent on the presence of parasites or embolic processes in the cerebral vessels. (See especially Case VII.)

The most striking pathological feature of the case is the massing of pigment in the kidneys, especially in the cells of Henle's loops. A careful review of the microscopic studies of the viscera in malarial infection, which is believed to be fairly complete, fails to show the report of any similar condition in uncomplicated malarial fever. In the studies of Bignami,[1] Guarnieri,[2] Marchiafava and Celli (1887),[3] Kelsch and Kiener,[4] Stieda,[5] Bastianelli (1894),[6] Barker,[7] Benvenuti, Thin,[8] and others, the number of parasites and deposit of pigment in the kidneys are described as moderate. This general rule is explained by Bignami by the rapid renal circulation. The condition in the present case appears to resemble that found in the kidneys in hæmoglobinuric malarial fever, in some cases of which large deposits of pigment have been found, but differs from them in the peculiar distribution of the pigment, and in the absence of hæmaturia.

On first observing the pigment deposits in the epithelial cells I was inclined to regard them as the remnants of recent parasites, on account of the close resemblance of many of the pigment circles to those of the crescentic bodies, and from the presence of vacuoles about some clumps. Further observations on this and other cases, notably Case VII, led to the conclusion, however, that most of such pigment wreaths here and elsewhere have never been associated with parasites, but are derived from altered red cells. In the present instance this conclusion

[1] *Atti d. R. Accad. med. di Roma*, 1890; 1893.
[2] Ibid., 1887.
[3] Ibid., 1887.
[4] Maladies des pays chauds, Paris, 1889.
[5] *Centralbl. f. allg. Path. u. path. Anat.*, 1893, iv, p. 321.
[6] *Bull. d. R. Accad. med. di Roma*, 1893; 1894.
[7] *Johns Hopkins Hosp. Rep.*, 1895, v.
[8] *Lancet*, 1896, i, p. 1414.

is based upon the absence of any trace of the body of a parasite about the pigment clumps, the abundance of the clumps about the injected vessels of the medulla, and the crystalline form and peculiar arrangement of the grains of pigment in many of the clumps.

Case II.—*Estivo-Autumnal Malaria. Extreme Malarial Infection. Very large numbers of Parasites in Viscera, especially in the Heart Muscle. Rosettes in the Peripheral Blood. Extensive Pigmentation of Serous Membranes.*

J. B., 10 years. Went to Santiago in June, 1898, and remained about five weeks. During the last two weeks he felt feverish in the afternoon and complained of abdominal pain and diarrhœa. Never had a chill. The same symptoms continued during four weeks after his return to New York, when he took no medicine. Lately his chief complaints have been weakness and increasing pallor, but he has noticed no recent paroxysms. On August 15, he felt much worse and walked to the hospital with difficulty. When first seen he was apparently moribund but could answer a few questions as above. He soon became comatose. The pulse was very feeble, 124 per minute; temperature 101.6°; respiration 40. Extreme pallor and slight jaundice were noted. On physical examination the heart sounds were very feeble. The spleen was not palpable but the area of splenic dullness was enlarged. The *urine* was alkaline, sp. g. 1014; it contained a little albumin, but no casts were seen. During the night he vomited occasionally; delirium alternated with stupor; the pulse failed steadily; the temperature rose to 103.8° F., and he died 14 hours after admission.

Autopsy.—10 hours after death. *Externally*, extreme pallor, slight jaundice, no œdema. Subcutaneous tissues show a peculiar brownish yellow discoloration. *Lungs*, moderately congested and œdematous. *Heart*, size normal; muscle very flabby and pale; valves normal; chambers slightly dilated. *Liver*, surface and section of dark slaty color; outlines of lobules indistinct; size and consistence normal. *Spleen*, 1½ lbs., uniformly enlarged, of very dark chocolate color, pulp diffluent. *Kidneys* show signs of acute degeneration. *Gastro-intestinal tract* shows throughout a moderate catarrhal inflammation, but no hæmorrhages or erosions. *Serous membranes* all show small areas of black pigmentation. Peritoneum is of a dark slaty color and in the parietal pleura there are some large irregular patches of dense black pigmentation. Marrow of ribs and vertebræ is very hyperæmic and of dark chocolate brown color. The *blood* is very anæmic and of distinct chocolate tinge. The *brain* could not be examined.

Microscopic Examination.—Blood.—Smears taken on admission showed a large number of æstivo-autumnal parasites. Most were of the signet-ring form, without pigment, and all except the earliest stages were about equally represented. In a few of the larger rings one or two fine pigment grains were noted. Besides the rings there were a good many older forms with clumps of pigment. A few rosettes were found in the blood. The red cells showed the changes of a severe secondary chlorotic anæmia.

Heart Muscle.—The number of parasites in the red cells filling most of the capillaries is enormous. At many points nearly every red cell contains one or more organisms and the vessel appears to be distended with masses of infected red cells and pigmented leucocytes (Plate X, Fig. 7).

The forms of the parasites are about equally divided between non-pigmented rings, pigmented spheroidal bodies, and rosettes; but no crescents or distinct ovoids could be found. There are in the vessels a few pigmented endothelial cells of the usual appearance, and many richly pigmented leucocytes.

Most of the muscle cells contain a moderate deposit of large greenish yellow granules. There are no evidences of fatty or other form of degeneration of the muscle cells.

Lungs contain a great many richly pigmented phagocytes with parasites in all stages of degeneration. The capillaries are frequently distended and sometimes apparently occluded by masses of red cells, pigmented endothelial cells, leucocytes, and macrophages. The vesicles contain a few large pigmented cells. Parasites are not more numerous than in the peripheral blood.

Kidneys.—The capillaries everywhere contain a moderate number of red cells harboring parasites. Most of these are the non-pigmented, ring-shaped organisms. A great many richly pigmented spheroidal parasites were seen in the same situation as the rings, and a few segmenting bodies were noted. In no place were the capillaries distended or occluded by masses of infected red cells and pigmented leucocytes, as was noted in the heart muscle. Evidences of phagocytosis are moderately abundant and extremely distinct, especially in the glomeruli. The phagocytic cells are always either mononuclear leucocytes or endothelial cells, the tubule cells being free from pigment. The observed phases of the process included the inclosure of an entire red cell infected with an unpigmented ring; the inclosure of one or many pigmented spheroidal bodies; all stages of the destruction of the bodies of inclosed parasites; and finally the circles and clumps of fine pigment grains, which remain after the destruction of parasites and red cells. From one to twenty circles were counted in large macrophages lying free in the capillaries. The glomeruli and the capillaries of the medulla contain more pigmented cells and parasites than do the cortical vessels. There is a marked acute degeneration of the lining cells of the convoluted tubules, but no signs of chronic nephritis. The tubules are slightly dilated, and contain either casts, or coagulum, or fragments of epithelial cells.

Marrow.—The deposit of pigment is very marked but much less than in the liver and spleen. The pigment is found in the endothelial cells, and especially in the large and giant mononuclear cells of the pulp cords. In these cells all stages of the ingestion and destruction of parasites could be followed. The number of parasites within red cells is considerable and larger than in the liver and spleen.

In sections of ribs and vertebral bodies very few fat cells are present, the marrow being excessively cellular. Nucleated red cells are abundant and some are of slightly increased size. The eosins and giant cells are distinctly increased.

In smears of the expressed marrow the same conditions were seen distinctly. Minute intracellular rings are very abundant, many cells containing two, and some three, or four, such parasites. Twin parasites were found in the cells, also, in the form of large vesicular bodies, one-third the diameter of the cell, and sometimes showing one or two very minute

pigment grains, but beyond this size, no twin parasites could be found. Well pigmented parasites were always single, and no cell was found containing two well developed spheroidal bodies.

A few minute rings and a great many pigmented spheroidal bodies were found apparently extracellular, a position which I am inclined to refer principally to post-mortem and artificial processes. Rosettes were abundant, and in several which were considerably flattened 18 spores could be accurately counted. A moderate number of young and middle-sized crescents were seen.

The phases of phagocytosis were quite distinct. The great majority of pigmented cells, and the only ones containing excessive pigment deposits, were the large mononuclear cells of the pulp cords. The pigment was found in the form of discrete grains, or often in compact clumps, or circles. From one to thirty or more of these pigment-clumps were seen in the larger cells. In many instances the circle of pigment surrounded a vacuole somewhat larger than the body of a parasite. In heavily pigmented cells irregular masses of granular pigment were usually found in the perinuclear region and elsewhere. The parasites seen within phagocytes included a few non-pigmented rings and many faintly staining pigmented spheroidal bodies. Many stages of the degeneration of parasites were seen in the bodies of these cells.

The frequent appearance of a circle of pigment grains around a small vacuole is probably referable to the complete destruction of the red cell (Plate X, Figs. 1 and 4). Many of these large circles of pigment grains surrounded a central compact clump, as though the englobed cell had contained a pigmented parasite. A great many small spherical masses of fine pigment grains, identical in appearance with the pigment clumps of full grown parasites or rosettes, were seen, and traces of the bodies of the parasites were often detected about the clumps. Many discrete coarser grains and clumps without vacuoles or surrounding granules seemed to represent that pigment which had been absorbed from the plasma after its discharge from the parasite. Larger conglomerate masses of older pigment were abundant, but even in these masses a granular structure was usually visible.

Finally, in the marrow smears, the formation of peculiar *vacuolated leucocytes* could be traced from the smaller and medium-sized mononuclear cells of the marrow. In these cells the nucleus gradually lost its affinity for methylene blue and became indistinguishable from the cell-body. At the same time, in the nucleus and cell-body, hyaline globules developed and gradually increased in size until the entire cell was reduced to a coarsely reticulated faintly staining mass, identical in appearance with the vacuolated leucocytes seen in the circulation (Plate XI, Fig. 8). This process affected both pigmented and non-pigmented cells.

On the other hand no resemblance could be traced between any of the stages of these degenerating cells and the various forms of parasites to be found in the marrow, none of which, when admitting of positive identification in stained specimens, exhibited similar degenerative changes. *In the fresh marrow juice these leucocytes very closely resemble the forms of large vacuolated parasites described by some writers.*

Spleen. The black color of the spleen is found to be referable to very heavy deposits of pigment, lying in endothelial cells, large mononuclear cells of the pulp and sinuses, and leucocytes in the sinuses, and in parasites infecting red cells. The outlines of the sinuses are no longer visible, being choked by the influx of pigmented cells, or distended or ruptured thereby. The Malpighian bodies are much reduced in size. No foci of large cells free from pigment were to be seen in the pulp tissue.

The parasites are very numerous and of all forms except crescents, but the majority appear to be inclosed in various phagocytes.

Liver.—The pigment deposits are extremely rich and are found in the long swollen endothelial cells of the capillaries and in large mononuclear cells lying often apparently free in the capillaries, and some of which are probably leucocytes. The parenchyma cells are free from pigment. The number of parasites is very large. Most of them are inclosed within the above phagocytic cells, in which all stages of their degeneration are to be seen (Plate X, Figs. 1 and 2).

In the red cells a moderate number of rings, pigmented spheroidal bodies, and a few rosettes are seen. In the capillaries of Glisson's capsule the numbers of parasites within red cells are very much greater than in the intralobular capillaries. The chromatic network of the liver cells stains faintly with methylene blue and is very ragged and irregular. There are no signs of fatty degeneration. The cells usually contain a deposit of large greenish granules.

EPICRITICAL: Among the features of this case, attention may be drawn to the rather sudden termination after a period of comparative freedom from severe symptoms, in which respect there is nothing very anomalous; to the prominence of the cardiac symptoms; to the acute degeneration of the kidneys; and to the activity and extent of the phagocytic process. These features may best be considered in connection with the pathological conditions found in the viscera.

As the patient's condition had not led him to seek medical assistance until a few hours before his death, it must be supposed that the paroxysms immediately preceding the fatal attack were mild, as is also indicated by the further history obtained. Such pronounced heart weakness occurring early in the first severe paroxysm of the relapse would seem to class the case with those of acute cardiac failure in pernicious malaria. It was noted in the physical examination on entrance that the first sound of the heart was barely audible, and the pulse extremely feeble. The microscopic examination showed the presence of unusually large numbers of parasites in the visceral capillaries, the heart muscle containing an excessive proportion of them. All the small vessels in this locality were filled or distended with blood containing a colossal number of parasites in all stages of development, with an abundant admixture of pigmented leucocytes. A considerable

mechanical effect of such a massing of parasites in obstructing the circulation can hardly be doubted, although no signs of complete thrombosis of small vessels could be found. On the other hand, no inflammatory reaction in or about the vessels or in the muscle fibres or supporting tissue, no hæmorrhages, or evidences of degeneration or necrosis of cells, could be found. Yet if the mere presence of the bodies of parasites in a tissue is capable of exciting any such inflammatory reaction or causing cellular degenerations, this was an unusually favorable situation to demonstrate these effects. No such changes existing, their absence appears to furnish some evidence that the bodies of malarial parasites do not exert any marked local toxic influence, but that their local action is largely mechanical. Granting that a considerable disturbance of the heart results from the general toxic condition, it seems probable that the extreme cardiac failure observed in the present case resulted from the mechanical obstruction of the circulation by the masses of parasites in the capillary vessels, but the evidence that such a relation existed can hardly be claimed as demonstrative.

In the kidneys, on the other hand, where the effects of a toxic agent were very marked, the number of parasites was comparatively small, hardly exceeding that found in the peripheral blood. In these organs there were distinct signs of severe acute degeneration, referable, not to the presence of parasites, but to the general toxæmia.

The phagocytic process was extremely active in the liver, spleen, and marrow, where the great majority of parasites were englobed in phagocytic cells. The leucocytes were everywhere actively engaged in the process. In the kidney the majority of phagocytic cells were leucocytes. In the heart muscle, and in the connective tissues generally, the parasites were very abundant and were multiplying rapidly, unrestrained by any phagocytic tendency in the endothelial cells.

Case III.—*Estivo-Autumnal Malaria. Marked Cerebral Symptoms and Concentration of Parasites in Capillaries of Central Nervous System. Infection with a single well defined group of Parasites. Developmental stages fully apparent throughout a 48 hr. cycle. Severe degenerative changes in the cells of the Convoluted Tubules, with absence of parasites in the renal vessels. Capillary Varicosities in the Liver, with atrophy of parenchyma.*

B. T., 32 years. While in Cuba he had attacks of chills, fever, and diarrhœa, but partly recovered under quinine. On the transport the same symptoms returned. After arrival at Camp Wikoff he suffered from nearly continuous fever without chills, and the diarrhœa became more profuse.

On admission to the General Hospital, September 6, he was considerably emaciated, slightly jaundiced, and completely prostrated. He was given 2½ gr. of bimuriate of urea and quinine, subcutaneously, t. i. d., and

opium. He passed a restless night and the temperature remained high. At 10 A. M., September 7, examination of the blood showed a recent sporulation of parasites. The temperature fell steadily during the day but the patient became delirious in the afternoon, and comatose by night, and never recovered consciousness. There was a moderate rise of temperature on Sept. 8, while the coma deepened and the pulse gradually failed. Death occurred at 7 A. M., Sept. 9.

Blood Examination. September 7, 10 A. M., showed a great many æstivo-autumnal ring-shaped parasites, 2-3 μ in diameter, lying in shrunken red cells. Most of them are entirely free from pigment, but rarely one or more fine pigment grains may be detected. A great many of the rings are less than 2 μ in diameter. Multiple infection of cells is not unusually frequent. In the fresh condition the parasites exhibit rapid changes in outline but few distinct pseudopodia.

The red cells show the changes of a severe secondary anæmia with beginning changes in the size of the cells. The leucocytes are reduced in number. No eosins seen. No pigmented leucocytes.

Sept. 8, 10 A. M., the parasites are much less numerous. They are nearly all of larger size, 4-5 μ, and maintain the form of a ring with thickened irregular segments. A few show one or two fine pigment grains. The chromatin is variously subdivided and usually displaced from the periphery of the ring, being often found as a small group of very fine granules in the centre of the ring, or arranged in the form of a crescent or figure 8, or as an irregular mass or group of granules lying at some distance from the ring. No spheroidal bodies with compact pigment mass and no rosettes were seen in this case. Several hours were spent at various times in the study of these specimens and during that time two crescents were encountered. The unity of the group of parasites is to that extent imperfect.

In the fresh condition the formation of pseudopodia and the amœboid motion are very active, and the pigment grains show slight vibratory motion. The leucocytes are as before.

Autopsy.—4 hours after death. *Body* markedly emaciated, slightly jaundiced; no œdema. *Lungs* show old emphysema and hypostatic congestion. *Heart*, rather small; valves and muscle normal; pericardium distended with clear serum. *Spleen*, slightly enlarged, moderately pigmented, dark brown, rather soft. *Kidneys*, about normal in size, consistence reduced, capsules not adherent, surface smooth, cortex somewhat thickened, pale, markings regular but indistinct. *Stomach*, contains bile, otherwise negative. *Intestine*, normal. *Brain*, no increase of serum, no venous congestion, no dropsy of ventricles; cortex slightly brownish on section; no petechiæ.

Microscopic Examination.—Liver.—The liver cells are very fatty, and contain many coarse yellowish granules, some of which give the reaction of hæmosiderin. No necrotic foci were seen. In many lobules the cords of liver cells are partly or completely atrophic and the capillaries are much widened, forming a variety of cavernous tissue. These changes are of irregular distribution in the organ, being sometimes most marked about central veins, but more often affecting large irregular portions of lobules. Pigmentation of endothelial cells and of leucocytes is marked but not extreme. Parasites are very scarce, but a few small spheroidal bodies and minute rings could with difficulty be identified.

Spleen. Appearances similar to those of Case II; but very few parasites could be identified.

Marrow. The marrow of the vertebræ is very fatty. In the cellular cords, there is in places moderate proliferation, and the cells appear in compact masses. Generally, however, the cords appear normal or deficient in colorless cells. The pigment deposit is slight and no parasites could be identified. In smears of the marrow a few ring-shaped parasites within red cells were identified. The nucleated reds, eosins, and giant-cells are very deficient.

Kidneys.—The convoluted tubules are markedly dilated and filled with granular coagulum. The cells are flattened, or broken and degenerated, and nearly all contain great numbers of large and small light yellowish granules which give the Prussian blue reaction of hæmosiderin. The capsules of the glomeruli are considerably dilated. The capillary tufts contain a moderate number of pigmented cells and a few spheroidal parasites. In a few of the ascending limbs of Henle's loops there are a good many clumps of pigment lying within the lining cells, but this condition is not at all frequent.

Brain.—Throughout the cerebrum, cerebellum, medulla, and upper cervical cord, the capillaries contain a very large number of red blood cells harboring parasites. Most of these are small pigmented spheroidal bodies; some exhibit the large ring form with little pigment, and a very few rosettes were identified. The pigment deposit, outside of the parasites, is slight. A considerable number of capillaries were found completely filled and apparently occluded by masses of infected red cells, pigmented leucocytes, and swollen pigmented endothelial cells. In the same regions the small arterioles and all the larger vessels were almost entirely free from parasites. The ganglion cells everywhere show reduction in size, irregularity, splitting, or loss, of chromatic bodies. These changes are less marked in the large stichochromes of the bulbar nuclei than in the cerebrum and cerebellum.

Epicritical: Although further evidence is hardly required to demonstrate the fact that in many cases of pernicious malaria of the cerebral type the capillaries of the brain contain an excessive number of parasites, the present case is such a striking example of this condition as to be worthy of record.

The relation of the cerebral symptoms can apparently be closely connected with the development of the parasites as followed in the examinations of the blood. Sporulation occurred during the night of September 6, when the temperature was at its highest point, 104°. At 10 A. M., September 7, when the patient was extremely restless, the blood and presumably the brain contained a large number of small ring-shaped parasites. Delirium and partial stupor began on the same afternoon. At 10 A. M., September 8, when the patient was comatose, the parasites had markedly increased in size and many had retired from the general circulation. At this time it is reasonable to infer that the

increased size of the parasites and probably their increased numbers in the central nervous system had seriously impaired the capillary circulation. At death, 3 A. M., September 9, preceded for several hours by profound coma, the sections of the brain show that the majority of the parasites had reached their full development, some were segmenting, and many cerebral capillaries were occluded.

The presence of a single group of parasites, the development of which could be followed throughout the cycle, is one of the interesting features of the case. Sporulation appears to have been completed during the night of September 6, when the temperature reached its highest point, 104°. At 10 A. M., September 7, the blood contained a large number of rings nearly all under 3μ in diameter, with a single large chromatin body, and without pigment. These parasites appeared to have had at least 6-10 hours' growth. At 10 A. M., September 8, the parasites had increased in size, measuring about 4-5μ in diameter; numerous outgrowths had appeared on the circumference of the rings; the chromatin (Nocht's method) was invariably increased in quantity, subdivided, and irregularly placed, and a few parasites showed slight pigmentation. There were still no spheroidal bodies, with compact pigment, to be found, after 30 hours' growth. The patient died at 3 A. M., September 9, and the great majority of parasites found in the cerebral capillaries were of large size and abundantly pigmented, and a few rosettes were seen, indicating the approach of general segmentation at the end of 48-50 hours' growth. Judging from the appearance of the parasites found in the sections of the brain it would appear that about 6-10 hours' growth separated considerable numbers of the youngest from the oldest members of the group, although between a few individuals the intervals must have been much longer.

The severity of the renal lesion, with the absence of parasites in the renal vessels, also requires mention. The changes in the cells of the renal tubules were more advanced than in any other uncomplicated case of the series and appeared to be purely of the type of acute degeneration. The lining cells were markedly eroded and largely composed of a multitude of light yellow granules giving the reaction of hæmosiderin. This destruction of the lining cells caused the dilated tubules to be more or less filled with granular detritus, but there was no further evidence of an exudative process. The kidney was free from chronic changes. In the absence of parasites and of signs or causes of acute inflammation, this lesion must be referred to a toxic condition associated with the malarial infection.

The evidence of the present case, therefore, fully accords with the conclusion drawn from other cases of the series, that the usual renal lesions of pernicious malaria are referable to the effects of a toxic process and not to the direct action of parasites.

Case IV. *Estivo-Autumnal Malaria. Extreme Malarial Infection. Localization of Parasites in Bone Marrow. Infection with only one group of Parasites. Pernicious Anæmia.*

F. J., 27 years, private, U. S. Army. No important details of the history of this case were obtainable. It was learned that the patient had suffered severely from malarial fever in Cuba and on the transport, and was received at the hospital at Montauk in a precarious condition, dying a few hours afterward in spite of stimulation and subcutaneous injections of quinine.

Autopsy.—6 hours after death. *Body* very much emaciated, markedly jaundiced. *Lungs*, lower lobes œdematous; commencing hepatization of lower part of right upper lobe. *Heart*, flabby, pale; valves normal; pericardium distended with clear serum. *Liver*, of moderate size, almost black; gall-bladder distended with bile. *Spleen*, moderately enlarged, soft, black. *Kidneys*, slightly enlarged, surface smooth, capsule not adherent, cortex thick, yellowish, markings regular, indistinct. *Gastro-intestinal tract*, negative. *Peritoneum*, slate colored. *Marrow* of ribs and vertebræ chocolate colored.

Microscopic Examination.—Blood.—The only specimens secured were squeezed from the incised finger-tip at the autopsy, six hours after death. The red cells are extremely deficient in number and usually fail to form rouleaux. The plasma stains slightly. There are extreme differences in the size of the red cells, which vary from small microcytes to very large megalocytes; most are larger than normal. Nearly all cells contain an increased quantity of Hb. and many are polychromatophilic. Nucleated red cells are abundant and nearly all fall in the class of megaloblasts or gigantoblasts. There is a pronounced polynuclear leucocytosis (antemortem?), but eosinophile cells are scarce. Many leucocytes, both mononuclear and polynuclear, are pigmented. Comparatively few parasites could be found, and these were all small rings, as seen in the smears of the marrow.

Liver.—There is slight fatty degeneration of the liver cells; the chromatic network is irregular and indistinct, and the cells all contain large greenish granules which give the reactions of bile pigment. There are no areas of necrosis. The deposits of malarial pigment are abundant and very similar to those described in Case II, but the evidences of inclosure and destruction of parasites are very much less marked. Very few parasites could be identified.

Spleen shows the usual changes of acute malarial infection.

Marrow.—That of the ribs and vertebral bodies is very cellular. The hyperplasia affects principally the myelocytes, many of which show mitotic figures, other small mononuclear cells, and giant-cells, of which there are many groups of five, six, or more. No fat cells were seen. The sinuses

are gorged with red cells and pigmented leucocytes. Nucleated red cells are very abundant but no "islands" of these cells could be found. The pulp cords contain a great many heavily pigmented endothelial and large mononuclear cells. A few parasites were identified in the sections with difficulty.

In smears of the marrow, all stages of the inclosure and destruction of parasites could be followed in the large mononuclear and endothelial cells. The number of parasites found in these smears is enormous, and nearly all are the small ring-shaped forms without pigment. They are much more numerous in the marrow than in the blood smears or in any other tissues of the case, and much more numerous than in the smears made from the marrow of any other case of the series. Examples of multiple infection of the same red cell are very numerous, three and four rings being very frequently seen; five and six were sometimes encountered, and one cell was found containing seven distinct young rings.[5] In spite of the enormous number of parasites and the abundance of nucleated red cells, none of the latter were found infected. No crescents were seen. Of the nucleated red cells many are of normal size and appearance, but a great many darkly staining megaloblasts and gigantoblasts are present. The eosinophile cells are about normal in numbers, while the giant-cells are distinctly increased.

The *kidneys* show the lesions of acute degeneration similar to those of Case II, but the number of parasites in the capillaries is small, and no rosettes were seen. Pigmentation is moderate.

In the *heart-muscle* a few ring-shaped and a very few small spheroidal parasites were found.

The *gastro-intestinal tract* exhibited no lesions of importance; its wall contained a moderate number of small parasites without pigment.

The brain was not examined.

Epicritical.—In this case there are several isolated features of interest. The localization of an enormous number of parasites in the marrow of the ribs and vertebræ is a peculiarity not encountered in any other case of the series. It has appeared from smears and sections of the marrow of other cases that the activity of phagocytes in this tissue, as in the liver and the spleen, greatly reduces the numbers of parasites that can be demonstrated in smears of these tissues. This general rule, first formulated by Guarnieri and Bignami, has applied in all other cases of my series, but in the present instance no large number of parasites was demonstrated in any other tissue except the marrow, where the excessive pigmentation is further evidence of unusual activity of the parasites in this locality.

Attention may here be drawn to the necessity of relying only upon smears of the tissues and not upon the examination of sections to

[5] Shown in Plate XXX, Fig. 2, in *Journal of Experimental Medicine*, 1901, v

determine the number of parasites present in tissues which are actively engaged in phagocytosis. Although formalin as used in fixing the tissues proved to be a very superior agent for this purpose, it has always seemed to me difficult and hazardous to attempt to identify parasites in stained sections of the liver, spleen, or marrow, and in many instances where none could be identified in sections, smears of the tissues revealed their presence in abundance. In the kidney, heart-muscle, brain, and all tissues where phagocytosis is less active, the results of examination of stained tissues appear to be fully reliable, especially if Nocht's method is used.

A further point of interest in the present case is the evidence pointing to the existence of a single very compact group of parasites in the blood and viscera. The forms found in the blood and in the smears of the viscera included only the smaller non-pigmented ring-shaped parasites. Neither pigmented spheroidal bodies, nor rosettes, nor crescents, were anywhere seen. This condition is the more remarkable since the pigmentation of the viscera, the grade of anæmia, and the general condition of the patient indicated a somewhat prolonged course of the infection.

The changes in the blood in this case are of special interest, illustrating the rapid development, as a result of malarial infection, of a condition identical in morphological character with that of primary pernicious anæmia. As the first cases of malaria among the troops at Santiago developed in the second week of July, this patient, dying on September 10, could not have been ill longer than eight, or possibly nine, weeks. Yet in this period a condition of the blood was established indistinguishable from that of primary pernicious anæmia, including the abundance of large megalocytes with excess of Hb. and the presence of many megaloblasts. Even the smaller red cells exhibited an apparent excess of Hb. Apart from the lesions directly referable to the growth of malarial parasites, the changes in the marrow included a well-marked cellular hyperplasia affecting principally the myelocytes and other small mononuclear cells, and giant cells; disappearance of fat cells; increase in size of the nucleated red cells, the majority of which were megaloblasts; and disappearance of "islands" of nucleated red cells.

Case V.—*Estivo-Autumnal Malaria. Massing of Parasites in Kidneys. Acute Hæmorrhagic Nephritis. Casts Entangling Infected Red Cells and Pigmented Leucocytes in Discharging Tubules. Infection with a single very compact Brood of Parasites.*

M. S., female, aged 17, contracted malaria on Long Island, and after a short period of mild paroxysms was completely prostrated on Sept. 12, 1900. On admission to hospital, Sept. 15, the chief symptoms were prostration, restlessness, vomiting, and moderate œdema of legs. Temperature 104.2° F. Urine, of high color, sp. g. 1018, contained many epithelial cells, detritus, and many red blood cells. Diazo-reaction marked. Widal's reaction absent. Bowels costive. Restlessness and vomiting were partly controlled by sedatives. The vomiting of a round worm led to the administration of three full doses of santonin on Sept. 19. Mild delirium developed into coma on Sept. 20, and with failing pulse and diminished, bloody urine the patient died on Sept. 20. There were two complete remissions of fever on Sept. 18 and 20, but no chill occurred. *Clinical diagnosis*, typhoid fever and acute hæmorrhagic nephritis.

Autopsy.—By Dr. Otto Schultze, 14 hours after death. Body well nourished, rigor firm, slight œdema of legs. *Heart, lungs, stomach,* and *pancreas* normal. There was one ounce of straw-colored serum in the pericardium, and a few ecchymoses in the visceral pleura and pericardium. *Intestine*, ileum congested, lymph-follicles slightly enlarged; colon normal. *Liver*, without gross indications of malarial infection, was pronounced normal. *Brain* normal. *Spleen*, 16 oz., firm, dark red, with prominent Malpighian bodies. *Kidneys* weighed together 16 oz.; cortex much thickened, capsule free, markings obscured, color very light; medulla intensely congested, in places rusty; in cortex of right kidney a superficial anæmic infarct with surrounding hæmorrhagic zone, measuring 3 x 2½ cm. in area, and 3 mm. in depth.

No gross evidences of malaria were detected.

Anatomical diagnosis.—Acute hæmorrhagic nephritis.

Cultures from liver and spleen yielded Bacillus coli communis, but no growth of Bacillus typhosus was obtained.

Through a misunderstanding all the viscera except the kidneys were thrown away. The kidneys were hardened in 5 per cent formalin.

Microscopic Examination (Plate XIII).—The lining cells of the convoluted tubules exhibited a very advanced stage of degeneration of a peculiar type. The cells were greatly swollen, their outlines obliterated, so that the distended tubules were filled with a coarsely reticulated mass of cell-detritus in which pyknotic nuclei were irregularly scattered. The degenerative process was principally hydropic, little fat or stainable protoplasm being present (Plate XIII, Fig. 13). There were many minute foci of necrosis, especially in the right kidney. The infarcted area was in an early stage of coagulative necrosis, and throughout this area, especially in the hæmorrhagic zone, the vessels were distended with blood containing enormous numbers of small pigmented parasites. Many small vessels leading to the infarct were distended to two or three times their normal calibre by thrombi of infected red cells. The capsules of the glomeruli were distended with granular coagulum (Plate XIII, Fig. 13). Most of the cortical capillaries were collapsed by the distended tubules and free from blood and parasites. Throughout the medulla were many miliary hæmorrhages, and most of the capillaries were distended with red cells, most of which contained parasites, or with thrombi of pigmented parasites which

had nearly destroyed the red cells. The parasites were remarkably uniform in size, being full grown æstivo-autumnal forms with abundant pigment. A very few rings and rosettes were seen. The discharging and many higher tubules were distended with granular, epithelial, or blood casts. Some of these casts had entangled leucocytes, often pigmented, and infected red cells, while some infected red cells lay free in the lowest discharging tubules. From their low position in the tubules all of these casts must have reached the urine in at least moderate numbers. The remarkable massing of parasites in the renal capillaries is shown in the photographs (Plate XIII, Figs. 14 and 15).

Epicritical: The absence of microscopic examination of the other viscera, while greatly to be deplored for the sake of completeness in the report of this case, cannot seriously detract from the importance of the findings in the kidneys. Doubtless there were many parasites in the other viscera, but in the absence of gross evidence of their presence it is safe to conclude that these viscera were not the seat of any special localization of parasites. This conclusion is rendered more trustworthy from the fact that the single compact brood in the kidney was composed of full-grown forms which were richly pigmented.

Moreover, numerous miliary hæmorrhages, and thrombosis of vessels with infarction, are the usual mechanical results of the presence of enormous numbers of parasites in a tissue, and the great extent of these lesions in the kidneys throws this case in the same class with the comatose and choleraic cases, with localization of parasites in the brain and gastro-intestinal mucosa respectively. The extreme degeneration of the tubule cells seems in part referable to the general toxæmia of the disease, but its extreme degree and peculiar character were not seen in any other case of the series, and may, with considerable certainty, be attributed to the obstruction of the circulation by the thrombi of infected red cells. Since the discharging tubules contained, among a large number of casts, some entangling infected red cells and pigmented leucocytes, while a few infected red cells were found free in the lumina of the tubules, it seems possible that a diagnosis of such a case might be established during life from the examination of the urine, which should show marked diminution in quantity, considerable albumin and blood, many granular, epithelial, some blood casts, and *infected red cells* and pigmented leucocytes, both free and adherent to casts.

Case VI.—*Estivo-Autumnal Malaria (?). No Parasites found in Blood. Few Parasites and unusually little evidence of malarial infection found in the Viscera.*

B., age 32. Served with regiment in Santiago campaign and contracted malarial fever in Cuba. Stated that he had four attacks in Cuba, marked

by chills and fever. The last and longest illness lasted three weeks. Was treated always by quinine with good results, but on omitting treatment the disease promptly relapsed. Recently he had suffered from mild chills daily, with more continued fever. On Sept. 4, the day of admission, he had a slight chill at 8 A. M. and that afternoon the temperature was 101.6°; pulse, 80.

Physical examination was negative, the spleen not being found notably enlarged. The patient was not markedly emaciated or anæmic. All symptoms pointing distinctly to malaria the patient was given quinine, but next morning, September 5, he had another chill and the temperature rose to 104°. Quinine was then increased, and given subcutaneously in large doses. On September 8, there was a moderate chill and rise of temperature. The stools were now diarrhœal, the abdomen moderately distended, the skin slightly jaundiced, and as the fever did not yield to quinine the blood was examined.

Blood Examination. Sept. 8.—The red cells showed the changes of well marked secondary chlorotic anæmia. The leucocytes were reduced in numbers. No eosinophiles. No distinct pigmented leucocytes, but a few contained single grains of pigment apparently malarial. In spite of prolonged search no parasites could be found. This result was confirmed by several subsequent examinations of the same and other specimens. The dissolved blood in the proportion of about 1 to 10 of water was mixed with a broth culture of B. typhosus, yielding prompt and typical clumping.

On the evidence of a temperature resisting quinine, of typhoidal stools and abdominal symptoms, absence of parasites from the blood, and presence of Widal's reaction, the administration of quinine was stopped from September 9 to 10 inclusive. The temperature declined, but the other symptoms persisted and the patient grew steadily weaker.

Blood examination, Sept. 9, gave the same result as before, but the Widal test was not repeated. Sept. 10, temperature fell to 99.6°, but the patient's condition was not improved. Quinine was again administered subcutaneously. Sept. 11, abdomen more distended; diarrhœa continues; no eruption; patient at times mildly delirious; pulse very weak. Blood examination, Sept. 11, showed no parasites, but a few characteristic pigmented leucocytes were seen. Sept. 12, patient died. The diagnosis was at this time regarded as probably typhoid fever.

Autopsy.—10 hours after death. Body moderately emaciated, slightly jaundiced. *Lungs*, moderately congested and œdematous. *Heart*, left ventricle slightly dilated, wall rather thin, flaccid; old thickening of mitral and aortic valves. *Liver*, moderately enlarged, moderately dark brownish in color, but not slaty. *Spleen*, moderately enlarged, firm, dark red in color. *Kidneys*, enlarged, capsule free, surface smooth, cortex thickened, pale; markings regular but indistinct. *Gastro-intestinal tract*, shows no lesions of importance; Peyer's patches are not enlarged or congested; mesenteric lymph nodes are not enlarged. The stomach contains blackish fluid but there are no evidences of gastro-duodenitis. *Brain*, in the absence of cerebral symptoms, was not examined. *Marrow*, hyperæmic, but shows no gross indications of malarial infection. *Blood*, moderately anæmic, but not otherwise changed in color.

Microscopic Examination.—Spleen shows the usual changes of acute malarial infection. The deposit of pigment, although considerable, is much less than usual in fatal cases of malaria. Smears of the splenic pulp made at the autopsy showed the presence of a very few ring-shaped parasites in red cells. The pigmented leucocytes and endothelial cells are of the usual character in acute malarial infection.

Liver.—The deposit of pigment is rather less abundant than in the spleen and is limited to the leucocytes and endothelial cells. The liver cells show destruction of chromatic network, and a moderate deposit of greenish granules; some contain fat globules. In the smears from the liver, no parasites could be identified, but some atypical intracellular pigmented spheroidal bodies were noted which may have been malarial parasites.

Marrow.—The deposit of pigment is scanty. Fat cells are moderately abundant in the ribs, but the evidences of cellular hyperplasia are distinct. In the smears some typical macrophages englobing parasites in all stages of digestion, are found. A few infected red cells are seen. The nucleated red cells, eosins, and giant cells, are deficient in number.

In the other viscera the deposit of pigment was much less abundant than in most cases of fatal malaria, and no parasites could be found.

Epicritical:—While the data in this case must be regarded as inconclusive in important respects, the facts demonstrated render the case one of unusual interest and obscurity.

The most important feature is the absence of parasites from the blood, as repeatedly demonstrated, in a patient who nevertheless died with a typical temperature curve of fatal æstivo-autumnal malaria. The absence of parasites in this instance is not fully explained by the previous treatment with quinine, as in all other cases of my series in which all parasites disappeared promptly after quinine, the patient improved. Moreover, it is an almost invariable experience that cases of malaria that die without parasites demonstrable in the blood are more chronic cases complicated by severe anæmia or cachexia. All the other fatal cases of acute malaria seen at Montauk showed many parasites in the blood at death, although many of them had been treated with very large subcutaneous injections of quinine for as long a period as in the present case. Accordingly the absence of parasites from the blood, under the conditions existing, at once raised a doubt regarding the nature of the disease.

The possible existence of typhoid fever was completely set aside by the autopsy, unless one assumes an infection by B. typhosus without intestinal lesions.

Both the gross and the microscopic examinations of the viscera, however, as well as the clinical history, indicate as the cause of death an acute malarial infection in the fourth or fifth relapse, proving fatal

with very few parasites in the blood, which disappeared promptly on the administration of quinine. The present case therefore seems to fall properly in a rare and obscure class described by Marchiafava, Bignami, and Bastianelli[10] (1894), in which few parasites were found in the blood during an acute fatal attack of malaria, and in which no distinct gross signs of malarial infection were found at autopsy, the diagnosis requiring a microscopic examination of the viscera.

These cases occurred in July or late in the summer. They usually presented the rather typical clinical symptoms of pernicious malaria, especially the cerebral symptoms. On examination of the blood, however, even early in the disease, very few parasites (one or two in a single slide) or, later, none at all, were to be found, while the viscera showed no distinct gross lesions of a previous severe malarial infection. On microscopic examination of the viscera, pigment deposits were unusually scanty and few or no parasites were found.

These cases, according to the authors, are not to be classed with those in which the parasites gradually disappear in severe acute infections, none being found in the finger blood at death although the viscera exhibit abundant evidence of malarial infection; nor yet with those in which the parasites disappear after a long infection has spent itself. They believe that the fatal issue is rather to be referred to general debility of the patient, or to an unusually virulent infection, or to an occasional combination with sunstroke.

In my case the patient had not suffered from the campaign more than had many others, and although he had undoubtedly been living under very unfavorable conditions, this fact, though important, seems insufficient of itself to explain the unusual features of the disease. Neither was there any indication that the patient suffered unusually from the heat which prevailed at that time, even at Camp Wikoff. Finally, a virulent malarial infection usually produces very large numbers of parasites, severe anæmia, and heavy deposits of pigment, all of which were absent, at least in the fatal attack.

If the present case is to be accepted as one of pernicious malaria, and it is essentially similar to those of the Italian authors, the conclusions must then be drawn that æstivo-autumnal malaria may pass through four or five relapses without leaving marked deposits of pigment in the viscera and may end in a fatal attack in which very few parasites, which soon disappear under quinine, are to be found in the blood or viscera.

After referring to their own cases of this character Marchiafava

[10] loc. cit.

and Bignami take pains to state that after years of experience they believe that fatal malaria does not exist without parasites in the finger blood. The present case cannot be regarded as an exception to this rigid rule, and I would particularly point out the fact that the patient's blood was not examined until 72 hours after the beginning of treatment by subcutaneous injections of quinine.

Case VII.—*Fatal Malaria from Infection with Large Tertian Parasite (Golgi). Extreme Anæmia. Prolonged Coma without Parasites in Brain. Catarrhal Colitis. Hæmoglobinuric Malarial Fever.*

G. F., aged 32, private, U. S. Army, contracted malarial fever at Santiago in July, 1898, and had had repeated relapses with short intervals of improvement, when he had felt able to return to duty. Had often experienced severe shaking chills at somewhat irregular intervals. Had steadily lost flesh and become very anæmic. About Sept. 11, was again prostrated with severe chills and fever which failed to respond to quinine administered by the mouth or subcutaneously. On admission to General Hospital at Camp Wikoff, Sept. 13, the patient was comatose, and remained so until death at 3 A. M., Sept. 16. When seen by me, Sept. 15, the patient was comatose and moribund. The foregoing items of history were obtained from Dr. S. W. Allen, of Boston, who had seen the patient on admission, and to whom I am indebted for the opportunity of studying the case.

Autopsy.—8 hours after death. Body much emaciated, excessively anæmic, and slightly jaundiced. Chocolate-colored fluid exudes from mouth. *Lungs*, anterior portions anæmic, posterior much congested, moderately œdematous, and unusually dark in color. *Heart* muscle very flabby, of pale brownish tinge; valves normal. *Blood*, very watery, of slightly brownish tinge, coagulating feebly. *Liver*, not distinctly enlarged; of slaty color but not extremely dark; outlines of lobules very indistinct; gall-bladder distended with bile. *Spleen*, greatly enlarged, soft, but not diffluent; of typical chocolate brown color. *Pancreas*, small, dark salmon in color, rather soft. *Kidneys*, slightly enlarged, surface smooth, cortex thick, deep red, in places rusty, markings indistinct; organ very greatly congested. *Stomach*, partly digested in places, contains some dark bloody fluid. *Small intestine*, exhibits no abnormalities. *Colon*, lower third the seat of an intense catarrhal inflammation with superficial erosions, and swelling of solitary follicles. *Mesenteric lymph-nodes* are not enlarged. *Serous membranes*, slightly darkened and jaundiced, and contain slight serous effusions. *Marrow*, very hyperæmic, of chocolate brown color. *Brain*, white matter perhaps slightly darker than usual; dura and pia yellowish; no effusions, no petechiæ. Specimens preserved in 5% formalin.

Microscopic Examination.—Blood, Sept. 15. Smears contain a very large number of tertian parasites in all stages of development. These parasites are of large size, richly pigmented, except in the very young forms; their nuclei fail to stain by methylene blue, and the infected cells are much swollen and distinctly pale. A prolonged search, repeated on several subsequent occasions, failed to show the presence of any bodies that could be classed with the æstivo-autumnal parasite. All the ring forms were coarse,

usually pigmented, with a large achromatic spot (methylene blue), and the infected cells were swollen.

The red cells show the changes of a severe secondary chlorotic anæmia. There was no leucocytosis, but the eosinophile cells are not relatively numerous. A moderate number of pigmented leucocytes were seen.

At the autopsy, smears upon glass slides were made from the expressed marrow of the ribs, and from the splenic pulp.

Smears from the marrow contained an abundance of pigmented cells. The pigment is usually darker and finer than that seen in æstivo-autumnal infections. When occurring in clumps, the clumps are rather more compact, but most of the phagocytic cells contain more diffusely scattered pigment grains than are seen in æstivo-autumnal cases. Many rods and clumps, however, are indistinguishable from the æstivo-autumnal pigment. Very few infected red cells could be found, but there were a few small pigmented intracellular bodies staining faintly with methylene blue. Nucleated red cells, eosinophile cells, and giant-cells are distinctly deficient.

Smears from the spleen contain a great number of pigmented cells similar to those of the marrow. There are a large number of distinctly stained pigmented parasites of considerable size. Very few or none of the younger parasites seen in the blood could be identified in these smears. No vacuolated parasites could be found, but there was a considerable variety of small, vacuolated leucocytes, many of which were pigmented (Plate XI, Fig. 8).

Spleen.—There is extreme pigmentation of all the larger cells, the pigment showing the same characteristics as in the marrow and liver. No parasites could be identified. There is a well-marked cellular hyperplasia of the pulp tissue; the large and small sinuses are choked with pigmentiferous and other cells; the Malpighian bodies are reduced in size, and the blood content of the organ is increased.

Abdominal lymph nodes show moderate hyperplasia, slight pigmentation of endothelial cells, many mast-cells, but no parasites.

Marrow (rib).—The pigment deposit is very heavy, but few parasites could be identified. The cellular elements are very abundant and in places there seems to be some proliferation. There are marked congestion of all vessels and a few small hæmorrhages.

Liver.—There is extreme pigmentation of endothelial cells, macrophages, and leucocytes, but the parenchyma cells are almost entirely free from brownish pigment. The deposit of pigment in the former cells differs from that seen in æstivo-autumnal cases, in being finer and more diffuse (Plate X, Fig. 3). Although there are many cellular inclusions in the phagocytes, no parasites could be positively identified. The liver cells are not fatty, but the chromatic reticulum is faint and irregular, and many cells contain coarse greenish granules. A few small foci of necrosis were found, in the neighborhood of which dilated capillaries filled with large phagocytic cells were sometimes noted.

Kidney.—The blood content of the organ is enormously increased. All the large vessels are gorged with blood, the intertubular capillaries in numerous small areas are greatly dilated and distended with blood. The capillary walls are usually intact, but in many places there are small hæmorrhages into the tubules. Everywhere, especially in the medium-

sized and larger vessels, there are peculiar collections of pigment to be described later. The quantity of ordinary malarial pigment is not large.

The cells of the convoluted tubules show advanced stages of degeneration. In the less congested areas the cells are moderately eroded, and contain a large deposit of fine yellowish granules of hæmosiderin. Near the hæmorrhages and between markedly dilated capillaries, the tubule cells are often necrotic, the nuclei fail to stain, and the protoplasm is broken up into shiny opaque globules staining with eosin. Sometimes the entire cellular lining is fused into a homogeneous opaque mass entirely detached from the membrana propria. In some lining cells and in some tubules there are a few pigmented parasites, which are also to be seen in the capillaries.

Lungs.—All capillaries contain an enormous quantity of granular and crystalline pigment in leucocytes, endothelial and epithelial cells, and free in the vessels. No parasites were identified.

Stomach and *small intestine*, negative. The inflamed *colon* shows intense congestion of mucosa, exfoliation of epithelial lining in places, moderate pigmentation of leucocytes and of endothelial cells. No malarial parasites identified and no Amœbæ dysenteriæ found.

Heart muscle.—In the capillaries of the heart muscle there are a good many richly pigmented leucocytes, considerable deposits of crystalline pigment, but very few parasites could be found, either in the red cells or leucocytes, or in the plasma. There is considerable artificial separation of muscle cells in some places, but the tissue shows no evidence of inflammation nor the cells any marked signs of degeneration. Many of them show a deposit of greenish granules about the nucleus. There are a great many huge mast-cells in the endomysium.

Brain.—Throughout the cortex, medulla, and cerebellum, there is the usual hyperæmia, but the great majority of all vessels are entirely free from pigment and parasites. In a good many capillaries an isolated, pigmented, intracellular spheroidal body is found, of slightly larger size than those seen in æstivo-autumnal cases. The pigment in these bodies usually appears in coarse dark grains. In a very few capillaries, there are masses composed of a few pigmented spheroidal parasites, pigmented leucocytes and swollen pigmented endothelial cells, capable of partly obstructing the circulation in the capillary, but these obstructed vessels are so scarce that it seems hardly possible to refer the cerebral symptoms to this condition.

There is a uniform reduction in the size and number of chromatic bodies in the ganglion cells and considerable post-mortem clouding.

Epicritical:—While the statement of Italian authorities has long held true that no autopsy has been reported in a case of malaria with infection by the large tertian parasite, as the infection is never fatal, the present case requires a modification of this view, offering an instance of fatal uncomplicated malaria with infection by the large, and so-called "benign" tertian parasite, demonstrated in the blood during life in large numbers and without admixture with other varieties, and indicated further by the somewhat peculiar character of the pigment deposits in the viscera

The above claim of the Italian observers, although based on a very wide knowledge and experience, must be regarded as too sweeping, since almost any condition may occasionally prove fatal through accidental complications. Thus, Barker[11] has reported a case of tertian malaria with autopsy, in which death was referable to general infection with Streptococcus pyogenes.

In another case seen by me at Montauk, death occurred in the third relapse when the blood contained a great many large tertian parasites, and no other organisms were found after repeated and prolonged examinations. Death in this case was undoubtedly due to the growth of this usually benign parasite, but in a subsequent review of one of the specimens, a single crescent was encountered, necessarily throwing the case in the class of double infections, according to the present theory of malarial species, but not seriously vitiating its value as a proof that the large tertian parasite is occasionally malignant.

The severity of the anæmia in the present case also deserves mention, in view of the general rule that the large tertian parasite is less active in impoverishing the blood than the malignant tertian. Although the Hb. was not estimated nor the cells counted, the pallor of the patient, the anæmia of the viscera, and the watery condition of the blood in the large vessels indicated a state of anæmia which was not exceeded in any other case of the series.

The prolonged coma, with very few parasites and very little pigment in the brain, classes this case with others which indicate that profound cerebral disturbance in acute malaria is not necessarily connected with the localization of parasites or pigment in the cerebral capillaries.

The catarrhal colitis and its cause are a feature of interest in this case. The microscopic examination failed to reveal any evidence that the malarial infection was directly concerned in this lesion, as few parasites and little pigment were found in the wall of the colon.

The character of the pigment in the case deserves special attention. As already mentioned, its occurrence in finer and darker grains more diffusely scattered throughout most phagocytes, especially in the hepatic endothelium, appears as a feature which may serve to distinguish the deposits of tertian from those of most æstivo-autumnal infections. Throughout the viscera, in the small and medium-sized vessels and in some capillaries there were deposits of a peculiar form of pigment somewhat similar in color to, but different in form from, that elabor-

[11] Op. cit.

ated by the malarial parasite. Sections of some of the injected vessels appeared almost black from deposits of this material. On examination this pigment appeared in the form of fine or coarse grains, or short rods, or short acicular crystals of dark brown color. It was usually attached to the surface of the red cells, but much was free in the plasma. Various stages of its formation could be followed in the blood vessels of the kidney, pia mater, and heart-muscle. In the earliest stage noted the red cells were found to be fringed with a row of small dark granules, while the body of the cell exhibited a faint yellowish-brown tinge. This appearance was often limited to the central portions of the mass of blood in the vessels, while adherent to the walls were leucocytes containing malarial pigment of the ordinary character (Plate XII, Fig. 11).

When more abundant, the pigment grains were usually of larger size and in quantity sufficient to fill the interspaces between the red cells. In this case the section of the injected vessel sometimes appeared opaque and brownish. Usually the deposit was very abundant and the red cells were covered with rod-shaped granules or short acicular crystals lying in or on the cell, or in the plasma (Plate XII, Fig. 12). Such cells sometimes exhibited a diffuse dark-brown color. Radiating groups of finer acicular crystals were commonly found in the richer deposits. In the kidney and heart muscle many arterioles were almost black with the deposits. No parasites were found in any of the red cells affected by the process. The bodies of the leucocytes were usually free from pigment grains of this character, but many were surrounded by numerous crystals, especially in the capillaries.

The distribution of the deposits was peculiar. They were found most abundantly in the heart muscle and kidney; the earlier stages were noted in the lungs, pia mater, and abdominal fat and lymph nodes; a few crystals were seen in the marrow; while the liver and spleen, containing an unusual quantity of ordinary malarial pigment, failed to show any trace of this crystalline variety.

These extensive deposits of pigment which were found in nearly all parts of the general circulation seem, without doubt, to be referable to a greatly increased globulicidal action of the plasma, and taken in connection with the renal lesions, indicate that the patient was suffering from that extreme form of destruction of blood which characterizes hæmoglobinuric malarial fever.

Case VIII.—*Estivo-Autumnal Malaria. Amœbic Colitis. Extreme Malarial Infection. Absence of Pigment and Parasites from the Wall of the Colon. Extensive Growth of Amœbæ.*

J., aged 21, private, U. S. Army. When received at the General Hospital, Sept. 9, on his return from Santiago, the patient was too ill to give an account of himself. He was then in a typhoid state, extremely emaciated, with incontinence of fæces, and mild delirium. Temperature 103°, pulse feeble. The temperature fell to 97° in the course of 48 hours, but the patient showed no tendency to improve. He was given moderate doses of quinine by mouth, and the diarrhœa was imperfectly controlled by astringents. The condition was at all times hopeless and he died Sept. 19.

Autopsy.—Two hours after death. Body extremely emaciated and anæmic. *Lungs*, show areas of œdema and hepatization. *Heart*, no gross changes. *Liver*, not enlarged; color very dark brownish, slaty; outlines of lobules indistinct; gall-bladder distended with bile. *Spleen*, very large, rather firm, of very dark chocolate color. *Stomach*, small, contracted. *Small intestine*, negative. *Colon*, lower third the seat of numerous ulcers of moderately large dimensions; bases and edges necrotic and undermined, covered with bloody necrotic material; vicinity in some areas covered with fibrin. Fæces greenish gray in color; semi-solid; contain little mucus; microscopically, they contain a little pus and epithelium, many bacteria, many fat crystals, no pigmented leucocytes, a little food detritus. No amœbæ could be found. *Mesenteric lymph nodes*, much enlarged, hyperæmic, very slightly pigmented. *Marrow*, hyperæmic, of chocolate tinge. *Blood*, watery; shows a chocolate tinge.

Microscopic Examination.—Spleen. Shows the usual lesions of active malaria.

Liver.—There are advanced degenerative changes in the liver cells. The deposit of pigment in various cells is extremely abundant. A moderate number of pigmented spheroidal parasites in the red cells, free in the capillaries, and englobed in macrophages were identified.

Marrow.—In the marrow of the ribs there is an abundant deposit of fresh pigment and several pigmented parasites were identified. Nucleated red cells, eosins, and giant-cells, are deficient.

Blood.—At autopsy found to contain a moderate number of ring-shaped æstivo-autumnal parasites. Red cells showed the changes of severe chlorotic anæmia. There was a moderate polynuclear leucocytosis.

Colon.—The ulcers extend through the mucosa, often to the muscularis, and are floored by a partly necrotic tissue infiltrated with pus and blood. Muscularis is often infiltrated with rows of large round cells. There is very little attempt at healing, and the process seems everywhere to be advancing.

Amœba dysenteriæ is found in considerable numbers in the submucosa. The amœbæ are most abundant beyond the edges of the inflamed areas, groups of them being found where there are no signs of exudative inflammation. In the bases of the ulcers and in the necrosing portions they are absent or very scarce. Evidences of the malarial infection here are very scanty, most of the vessels being entirely free from parasites and pigment. Rarely an isolated slightly pigmented leucocyte is encountered, but no parasites could be found.

There is an extensive growth of bacilli, apparently post-mortem, in and on the ulcerating areas. No cocci could be found in the sections. The complete preservation of the amœbæ, and the absence of post-mortem

changes beyond the walls of the ulcers, as well as the absence of pigment, precludes the possibility that malarial parasites could have been present in the ulcers during life and disappeared very shortly after death (2 hours).

Epicritical.—The present case illustrates a condition rather commonly encountered among the sick at Camp Wikoff on their arrival from Santiago. In the present instance the two protozoan infections appear to have limited themselves strictly to their natural situations and to have had little influence upon each other.

The wall of the colon contained large numbers of amœbæ, while exhibiting almost no indications whatever of the coincident malaria. The liver, spleen, marrow, and peripheral blood, on the other hand, showed the changes referable to an extreme malarial infection quite as severe as that demonstrated in some cases of fatal uncomplicated malaria. If there is any special tendency of an inflamed and ulcerated colon to gather malarial parasites or pigment from the blood, it failed entirely to show itself in this case.

It may be specially noted that while amœbæ were very abundant in the wall of the colon, none whatever were found in the fæces although they were carefully examined under favorable conditions at the autopsy. It should be said that there was very scant mucus, though considerable bloody necrotic material attached to the fæcal matter.

Case IX.—*Tertian Malaria. Chronic Endocarditis. Disposition of pigment three months after subsidence of the active infection.*

J. H., aged 48 years. Had suffered considerably for two years past from dyspnœa on exertion, palpitation, attacks of bronchitis, and transitory œdema of extremities. In June 1897 he had a sharp attack of chills and fever, but recovered under quinine. These attacks were repeated at intervals during the summer but no acute attacks occurred during the winter of 1897-98. In June, 1898, the attacks recurred and the patient was admitted to hospital in a very debilitated condition. The spleen was much enlarged, the anæmia marked, and many tertian parasites were found in the blood. The last acute attack occurred early in July, 1898. Quinine was administered constantly and no further relapses occurred. Meanwhile the symptoms of endocarditis had steadily progressed and on October 11, the patient died.

Autopsy.—One hour after death, by Dr. John H. Larkin, to whom I am indebted for the material from the case.

Heart, much enlarged, chambers dilated, and the mitral and aortic valves markedly stenosed. *Lungs*, very emphysematous. *Liver* showed chronic congestion and fatty degeneration, but no gross indications of malarial infection. *Spleen*, much enlarged, firm, and unusually dark in color. *Kidneys*, the seat of chronic congestion and slight chronic diffuse nephritis.

Microscopic Examination.—Spleen. The Spleen shows moderate evidences of

chronic congestion, in the widening of the pulp sinuses. The Malpighian bodies are distinctly outlined. Most of the pigment is gathered in large conglomerate masses of dark coarse grains lying in and distending the endothelial cells and macrophages about the Malpighian bodies, or in the walls of the splenic arterioles, or in the septa and walls of the veins (Plate XV, Fig. 18). A moderate number of endothelial cells and macrophages in the pulp tissue contain a scanty deposit of yellowish discrete grains. The red cells in both liver and spleen are well preserved, and some contain pigment grains and bluish stained spheroidal bodies which cannot be certainly identified as malarial parasites.

Liver.—There are well-marked lesions of chronic congestion with dilatation of the capillaries in the centres of lobules, with atrophy of the adjoining cells, and fatty degeneration of central and peripheral cells. Most of the pigment is collected in very large intracellular masses either in the portal canals or in the centres of lobules (Plate XV, Fig. 19). A few endothelial cells throughout most lobules contain scattered grains of apparently fresh pigment. The liver cells appear to be entirely free from malarial pigment which is confined to the endothelial cells and large macrophages lying loose in the capillaries, or between the connective-tissue fibres of the portal canals. The pigment grains are usually coarse, dark, and thickly conglomerate, but less often are small, yellowish and discrete.

Marrow.—A few endothelial cells containing coarse black pigment grains were found.

Lungs.—Any malarial pigment present could not be distinguished from the anthracotic pigment of the septa and the fresh blood detritus in the epithelium of the vesicles, belonging to the chronic congestion of the organ.

Epicritical:—The present case is reported to illustrate the condition of the malarial pigment three months after the subsidence of the acute infection. It will be seen that this period had sufficed to remove the traces of malarial infection from most of the viscera, while in the chief depots of pigment, the spleen and liver, the pigment had been largely transferred from the parenchyma to the connective tissue structures, giving sections of these organs a very characteristic appearance (Plate XV, Figs. 18 and 19).

It is to be noted that the above period was not sufficient to enable all the endothelial cells of the liver and spleen to become entirely free from pigment, some of them still presenting the usual appearance of the phagocytes of acute malarial infection. It is possible, however, that these cells were further signs of a partly-suppressed growth of the parasite, uncertain indications of which were also noted in the presence of bluish-staining pigmented bodies found in a few red cells. In appearance the old pigment was considerably blacker than the fresh deposits. It was usually gathered in more compact and somewhat larger spheroidal masses. About the clumps the outlines of an enclos-

ing cell, often enormously distended, could nearly always be discovered, but some of the pigment appeared to be lying free in the lymphatic spaces of the connective tissue.

Most forms of pigment deposited in tissues undergo gradual solution in their inclosing cells, yielding, in the case of iron-holding pigment, a diffuse reaction of hæmosiderin (Ziegler, Thoma, Neelsen). When submitted to the test for hæmosiderin, the cells in the neighborhood of the pigment clumps often gave a more intense blue reaction than was found in the other portions of the liver and spleen. On the outskirts of the clumps the granules appeared very fine, translucent, and of a yellowish tint, but none of these granules gave the blue reaction.

Case X.—*Acute metritis. Purulent peritonitis, from infection with Streptococcus pyogenes. Extreme deposits of pigment in all viscera, closely resembling those of malaria.*

B. D., aged 25. There was no previous history of malarial infection. The patient had always enjoyed good health since childhood. Jan. 2, 1899, the patient was delivered of a healthy infant. She felt well until Jan. 6, when she was suddenly seized with a chill and fever, while the lochial discharge increased in quantity. The physician in charge regarded the condition as referable to malaria and administered quinine by mouth in large doses. The fever persisted and the chills were repeated, but there was no distinct periodicity in their occurrence. A digital exploration of the uterus was made on Jan. 7, and its cavity was found to be free from placental remains. A severe diarrhœa developed but yielded to opium, and was succeeded by constipation on Jan. 12. Jan. 13, another physician was called in, who found signs of general peritonitis, advising removal to hospital. He gave no quinine on Jan. 13 and 14, and it could not be learned how long this treatment had been continued. No local treatment had been used at any time. On admission to Roosevelt Hospital, Jan. 15, the patient was in collapse, with signs of general peritonitis and a moderate discharge from the uterus of chocolate-colored fluid of slight odor. The symptoms were entirely those of peritonitis from puerperal infection. Temp., 104°; pulse, 160; respiration, 38. The urine was acid, sp. g. 1024, contained a trace of albumin, pus, blood, many vesical and renal epithelial cells, but no casts. Death followed a few hours after admission.

Autopsy.—By Dr. Eugene Hodenpyl, 20 hours after death. Body of fairly nourished anæmic and jaundiced subject. No œdema. *Lungs*, much congested, œdematous, with a few small areas of hepatization. *Heart*, muscle pale, flabby, valves normal, chambers slightly dilated. *Liver*, size slightly increased, section smooth, rather darker than usual, outlines of lobules indistinct. Gall-bladder contains 2 oz. of dark bile. *Spleen*, moderately enlarged, very soft, of well-marked slate color. *Kidneys*, slightly enlarged and softened, markings swollen, indistinct; cortex irregularly congested and mottled. *Peritoneal cavity* contains a half-pint of sero-purulent fluid and is everywhere highly inflamed and granular. *Gastro-intestinal tract*, no lesions. *Marrow* of ribs and vertebræ, very hyperæmic, and of dark brown color.

Brain, pia slightly œdematous, vessels gorged with blood, brain tissue jaundiced; no ecchymoses or other abnormalities noted. *Uterus*, slightly involuted, wall rather friable, pale; mucosa deeply congested, of dark red color, covered with a thin chocolate-colored layer of mucus, blood and pus. No remnants of placental tissue to be seen in the cavity of the uterus. Cervix moderately lacerated. No thrombi or pus in vessels of broad ligament.

No cultures were made from the peritoneum or uterus. In smears of the pus from the peritoneum and from the uterine discharge large numbers of cocci in short chains were found. In sections of the wall of the uterus large numbers of cocci in short chains were found, but only on the surface or superficial portions of the mucosa. These cocci were identical in morphology with the ordinary form of Streptococcus pyogenes seen in pus.

Microscopic Examination.—Spleen. There is a very abundant deposit of pigment in the endothelial cells and macrophages of the pulp. In the sinuses there are many richly pigmented leucocytes. The pigment is mostly in the form of grains, short rods, or longer acicular masses. It is very frequently arranged in the form of a small mass of fine brownish grains or crystals. In some places it is more compact and darker. About some of the masses there are minute vacuoles. No malarial parasites could be seen within the red cells, but many contain one or more coarse pigment grains.

Smears of the spleen show abundant pigment, principally in the large mononuclear cells. The pigment is principally granular, but many short crystals are seen. No malarial parasites could be identified.

Liver.—The pigment in the liver is very abundant, occurring in the same form as in the spleen. The liver cells contain considerable pigment in the form of granules, spheroidal or wreath-shaped masses, or elongated rods. Many of the elongated, rod-shaped collections lie in small clear spaces within the liver cells, which contain also much greenish granular bile pigment and show moderate fatty degeneration. No malarial parasites were seen.

Kidney.—There are evidences of acute degeneration of the tubule cells. The convoluted tubules and adjoining capillaries are nearly free from pigment. In the glomeruli are a few pigmented endothelial cells and leucocytes. In the medulla and medullary rays, many of the lining cells contain numbers of wreath-like masses of granular and crystalline pigment. The adjoining vessels are injected with blood containing many pigmented leucocytes, apparently free pigment granules, and a few pigment wreaths, but no parasites.

Heart-muscle. A considerable number of pigmented leucocytes are present in the capillaries but no parasites could be found.

Lungs contain a rich deposit of pigment lying principally in the capillary endothelium and outlining the vesicles in a pigment network. Some of these endothelial cells or macrophages are of extreme length and contain small wreaths of brownish pigment granules and crystals. Other cells show larger, denser clumps of pigment. In the pneumonic areas the exudate is composed largely of polynuclear leucocytes among which lie many pigmented cells.

Uterus.—The large superficial uterine sinuses contain blood-clots in many stages of organization. Over the inner surface of the uterus there is a thin

layer of pus and granular detritus entangling many pigmented leucocytes and a great many cocci in short chains. The evidences of exudative inflammation are limited to the superficial tissues. In the deeper tissue there is an extremely abundant deposit of wreath-shaped masses of pigment within capillaries, endothelial, large and small mononuclear, and connective-tissue cells. In many foci, especially in the neighborhood of large vessels, the pigment is quite as abundant as in the spleen. The sinuses and capillaries are injected with blood containing a large amount of free pigment and many pigmented leucocytes (Plate XI, Fig. 10). No malarial organisms could be identified. No smears were made from this organ.

Marrow.—The marrow sections and smears contain a moderate number of pigmented macrophages and endothelial cells. No infected red cells could be found. Nucleated red cells are abundant; the myelocytes are in excess; the giant cells are slightly increased; there are very few eosinophile cells.

Brain and Medulla. A few pigmented endothelial cells and leucocytes were found, but no parasites.

Blood smears were made at the autopsy from the finger tip, mesenteric veins, and cerebral sinuses. The red cells exhibited the changes of a severe chlorotic anæmia with leucocytosis. A few pigmented leucocytes were seen. In spite of prolonged and repeated search no parasites could be identified in the blood smears.

The *blood-vessels* throughout many of the viscera contain an abundant deposit of pigment in granular and crystalline form, lying outside of and adherent to the red cells. Many of the red cells have a peculiar brownish tinge. Much pigment lies within leucocytes and endothelial cells, and the tissues in the neighborhood of such vessels sometimes contain a moderate deposit of pigment granules and crystals.

Epicritical: The present case is inserted in this connection for the purpose of illustrating the great similarity which the pigment deposits of septic conditions may show to those of malaria.

In this case the possibility of malarial infection may be ruled out on the previous history, the distinct onset and course of ordinary septic metritis and peritonitis, by the failure of quinine to control the disease, and by the absence of parasites from the blood and viscera. On the other hand, the gross appearance of the spleen was identical with that of acute malarial infection, the liver and marrow exhibited a distinct brownish tinge, while the microscopic appearance of the pigment deposits was in many respects identical with that of malarial pigmentation.

On comparing these deposits with those of malaria some differences were to be noted. In the case of septicæmia the pigment grains were more often distinctly crystalline, and large, spheroidal, homogeneous black grains were more abundant than in acute malarial cases, while the fresh, yellowish, finely-granular pigment was much less abundant. The concentration of pigment deposits in the tissue about blood-vessels

was a somewhat characteristic feature. The wall of the uterus contained nearly as much pigment as the spleen. The pulmonary exudate contained an abundance of pigment, but in malaria pulmonary exudates have always been found nearly free from pigmented leucocytes. In the present case a considerable number of liver cells contained large and small vacuoles partly filled with brownish acicular crystals. In malaria this condition of the liver cells is rather rarely, though occasionally, encountered. In both cases the crystals fail to give Gmelin's test, they dissolve in ammonium sulphide, but not in ether, chloroform, or carbon bisulphide. The condition of the blood in sections of vessels showed that extensive destruction of blood cells was in progress throughout the viscera in the present case, and offers an ample source for the visceral deposits.

Nevertheless it would be impossible from a study of the pigment deposits alone to deny the coexistence of malarial infection, for as is shown in the description of other cases a similar destruction of blood may and in some degree frequently does accompany malarial infection. In Case VII the pigment deposit resulting from solution of blood cells completely overshadowed that derived from the malarial parasite.

The destruction of blood cells in various septic conditions may be confidently referred to the increased globulicidal action of the plasma which has long been known to characterize these conditions.

From the examination of this and many other cases I have become convinced that it is frequently impossible to distinguish between the pigment deposits of malaria and those resulting from increased globulicidal activity of the serum in other diseases. It follows that it is rarely, if ever, possible to establish the diagnosis of malaria from the presence of pigment deposits in the viscera. For in various other diseases there may be a deposition of pigment which is practically indistinguishable from that of malaria in color, form, intracellular position, general distribution, and chemical reactions. Moreover the slate color of the spleen in this case and the dark brownish tinge of the liver indicate that these are by no means pathognomonic gross signs of malarial infection. A further important inquiry concerns the extent to which the solution of blood cells in septic conditions is referable to post-mortem action of the globulicidal serum.

PART II.

I. TECHNICS.

Fixation:—The majority of investigators have preferred alcohol as a fixative agent in the study of tissues in malaria, placing small pieces in weak solutions for preliminary fixation, or using strong or absolute alcohol from the first. There seems to be nearly complete agreement that this is the best method to employ in this particular field. In addition to alcohol, a great variety of metallic solutions have been used with varying success, but none of them has been urgently recommended over alcohol.

In the present cases the fixatives used were alcohol, 80%, Lang's fluid, 1%, aqueous bichloride, and formalin 5-10%. The bichloride solutions may at once be discarded for the present purpose, on account of their tendency to leave metallic precipitates. In other respects they yielded good but not superior results. The tissues fixed in alcohol 80% gave satisfactory preservation of red cells and parasites, but less successful results as regards the tissues, which suffered the usual shrinkage. Hardening in formalin, 5-10%, proved most satisfactory. Shrinkage did not disturb the sections, the preservation of red cells and parasites was even better than that by alcohol, while the proved excellence of formalin as a fixative for general cytological study was very constantly apparent. Formalin seems to have a distinct advantage over some other fixatives in precipitating and rendering insoluble the pigments derived from dissolving red blood cells.

Staining:—Malarial parasites and their derivatives are well demonstrated in thin sections by staining 5 min. in 1% aqueous methylene blue, gently warmed, followed by moderate decolorization in strong alcohol, and clearing in oil of cajeput. This staining fluid may perhaps be slightly improved by adding a trace of alkali (.01% caustic potash), as used by Guarnieri. This method is practically that of Nissl and gives excellent demonstrations of changes in visceral as well as in ganglion cells.

Intracellular parasites are somewhat more deeply stained if the sections are previously treated for one hour with a moderately strong solution of hæmatoxylin, which also intensifies the nuclei of tissue cells. The use of hæmatoxylin clearly demonstrates the nuclei of the young and the segmenting parasites which are faintly or not at all stained in sections by methylene blue. *Amœba dysenteriæ* in sections stained by hæmatoxylin and methylene blue is very clearly demon-

strated, and may readily be identified by low magnification (Leitz No. 3). The nucleus of this protozoon fails to stain by methylene blue, but is sharply developed by hæmatoxylin. On several grounds, therefore, the combination of hæmatoxylin and methylene blue may be strongly recommended for the present purposes.

Attempts were made to stain the nucleus of the malarial parasite in tissues by the methods of Romanowsky, Ziemann, and Nocht. The results were not successful in demonstrating the chromatin, but Nocht's method brought out the body of the parasite very much better than any other method employed.

Very striking demonstrations of *Amœba dysenteriæ* were obtained by the application of this method to thin sections of the colon. These sections were stained for 24 hours and slightly decolorized 10-15 min. in 95% alcohol. The nucleus of the amœba then appears deep red, the body blue, the vacuoles are distinct and their contents variously stained.

In the study of pigment deposits in the viscera hæmosiderin was demonstrated by the common method, that of Perls. Sections were placed for one hour in 1% watery solution of potassium ferrocyanide and mounted in glycerine containing 1% hydrochloric acid. A useful procedure may sometimes be found in dissolving the malarial pigment by ammonium sulphide, which at the same time blackens the granular or diffuse hæmosiderin.

Formalin-fixation is a reliable means of identifying bilirubin, which is converted to the green biliverdin, but under the microscope the green tinge is not marked and the biliary derivatives may be mistaken for the yellow hæmosiderin granules.

I would strongly recommend the employment of smear preparations of the viscera, treated as blood specimens,[12] for the study of minute cellular changes, phagocytosis, and the demonstration of parasites. In the actively phagocytic viscera—liver, spleen, and marrow—it is often hazardous to attempt the identification of parasites in sections, whereas the examination of a smear made from these tissues furnishes convincing evidence of the number and type of parasites present.

II. THE VISCERAL LESIONS.

LIVER.

The *gross lesions* of the liver were usually but not always indicative of malarial infection. The organ was generally slightly swollen, but

[12] For the methods of staining smear specimens, see Ewing, Malarial parasitology, *Journal of Experimental Medicine*, 1901, v, p 429

this fact was not distinctly apparent at the autopsies, and in one case (III), with marked atrophy of liver cords and development of cavernous tissue, the liver was reduced in size. The consistence of the organ was usually slightly reduced. Fatty changes were seldom apparent to the naked eye. The pigment deposits were in the present cases always sufficient to give to the section of the organ a slightly brownish tint, but this change was sometimes far from characteristic and failed to be noted. In other cases the liver was slaty or black. The outlines of lobules were generally indistinct. Distension of the gall-bladder was commonly seen. That fatal malaria may leave inappreciable changes in the gross appearance of the liver is evident from Case VI, and especially from the cases of Marchiafava and Bignami in which very few or no parasites were found in the blood at death, and in which no microscopic evidences of a previous severe infection were found. It appears also that the pigmentation of the liver may be very slight when the parasites are massed in the intestinal mucosa.

The *microscopic examination* of the liver in the foregoing cases showed that the lesions varied but little from those described in acute malarial infection by Guarnieri in 1887, and Bignami in 1890.

Parasites, in the majority of cases, were comparatively scarce. They were usually englobed by phagocytic macrophages and endothelial cells, together with red cells, leucocytes, and pigment, and in these situations they almost invariably presented evidences of degeneration and solution. They were not positively identified in the liver cells. In Case II, dying just after sporulation of one of two or more very numerous broods of parasites, very large numbers of parasites were found in the liver, mostly within phagocytes and degenerating. In the other cases the hepatic phagocytes contained only scanty traces of the bodies of parasites, but many vacuoles and fresh pigment clumps. It is difficult to explain this difference except on the ground that the englobement of parasites in the liver is more active at certain periods of the cycle, or occurs intermittently, and that the destruction of englobed parasites may be completed very rapidly, i. e. within a few hours. Otherwise the livers of all acute cases with rich infection, ought to show abundant traces of the bodies of englobed parasites, which they did not show, with one exception, in this series of cases. It is to be noted that the exception was the only case of the series in which numerous rosettes were found in the tissues. There are several observations pointing to an increased phagocytic activity in the blood at the height of the paroxysm. Possibly the same rule holds in the liver.

However that may be, it is certain that the livers of acute cases with rich infection, exhibiting about an equal quantity of recent pigment, contain a very variable number of parasites in the early stages of destruction by phagocytes.

Among the active phagocytes could be identified the large mononuclear cells of the capillaries, endothelial cells, and leucocytes (Plate X, Figs. 1-6). There were often evidences of a fusion of large mononuclear cells, endothelial cells and leucocytes into large protoplasmic masses inclosing several infected red cells. Occlusion of capillaries by these masses or by many discrete cells was often observed, in the neighborhood of which the capillaries were sometimes found dilated.

Various lesions of the liver cells were observed. The normal liver cell stained by methylene blue, in sections and especially in smears, exhibits a coarse but regular and distinct chromatic reticulum. This reticulum was often found to be indistinct, its meshes uneven, and sometimes obliterated or displaced by various granules, indicating the changes of acute degeneration. Necrotic cells were rarely discovered in the present cases. I found many cells in advanced stages of degeneration, but few in which the nucleus was not demonstrable by hæmatoxylin or methylene blue, although the staining reaction was often faint.

Fatty infiltration of the liver cells was remarkably slight. In one case there were large areas in which the capillaries of irregular portions of lobules or of entire lobules were greatly dilated at the expense of the liver cords, which were markedly atrophic or had disappeared. The resulting appearance was that of cavernous tissue, as described in a few cases by Guarnieri, Bignami, Nepveu,[13] Lodigiani,[14] and Monti.[15] These dilated capillaries do not radiate from a central vein, as in the nutmeg liver of ordinary chronic congestion. Guarnieri refers this lesion to the disordered nutrition of liver and endothelial cells resulting from the obstructed circulation, partly also to direct pressure, conditions which existed prominently in my case. Bignami connects it especially with a previous necrosis of the liver cells. In the present cases these cells were not necrotic

The liver cells usually contained many large and small greenish granules of bile pigment. They were most abundant in Case VII, which exhibited externally the most intense jaundice, but were abundant in

[13] *Marseille médical*, 1894, xxxi, p. 649.

[14] Cited by Barbacci in *Centralbl. f. allg. Path. u. path. Anat.*, 1899, x, p. 64.

Case II, in which the jaundice was slight. That the intercellular bile capillaries were extensively occluded by pressure of swollen endothelial cells and macrophages in distended capillaries was evident, and to this condition the biliary pigmentation of the liver cells may be referred.

In all cases the liver cells presented a variable number of fine, light yellow granules which gave the reactions of *hæmosiderin*, while a diffuse reaction for hæmosiderin was obtained in some endothelial cells and in the connective tissue of the portal canals.

I was unable to find evidences of the hyaline transformation of the nuclei of liver cells described by Guarnieri, except in a few isolated instances. A variety of nuclear changes were observed in the liver cells which did not differ from those seen in other infectious diseases. Many nuclei were found in the process of direct division, while mitotic figures were rare. In some cases the evidences of regeneration on the part of the liver cells seemed to justify the use of the term "diffuse vicarious hypertrophy," employed by Guarnieri.

The portal canals were sometimes markedly infiltrated with round cells. Mast-cells were rather numerous in some areas, and sometimes these cells were pigmented.

While the present cases do not bear directly upon the question of cirrhotic processes in the liver following malaria, it was strikingly apparent that the infection had exhausted itself in producing vascular and cellular alterations, while there was an entire absence of the changes of beginning fibrosis.

SPLEEN.

The spleen was increased in size in all cases, the change being generally proportionate with the length and, to a less extent, with the severity of the infection. It was noted at Montauk that the spleens of the malarial cases were as a rule much smaller than those of the typhoid cases. The organ was usually much softened and sometimes diffluent. The capsule was tense. In one case (VII) the spleen was rather firm and dark red. In the color there was usually distinct evidence of malarial infection, but this was sometimes slight, and the spleens of cases of typhoid fever in malarious subjects often gave no gross evidence of considerable pigment deposits demonstrable microscopically. In all the frank acute malarial cases the spleen was moderately enlarged, soft, and of dark brown, chocolate, or slate color.

The most prominent *microscopic* features were the pigment deposits and the cellular hyperplasia and distension of the pulp cords.

It was always difficult to identify positively parasites in the sections whereas they were always found in larger numbers in smears of the pulp tissue. They appeared to be more abundant than in the liver, and the same description of their inclosure and destruction in phagocytes applies to both liver and spleen. The macrophages were much more numerous and of larger size in the spleen than in the liver.

In all cases the sinuses and cords contained an excessive number of cells. The evidence of proliferation of splenic cells was most apparent in the frequent presence of islands of 8 to 10 cells of small size, compact grouping, deeply staining nuclei, and free from pigment. The Malpighian bodies participate largely in this process, as their dimensions were sometimes increased, and their outlines often irregular, and fringed at times with these islands of young cells.

Marked obstruction to the circulation in the spleen must have existed from the distension of sinuses by macrophages, swollen endothelial cells, leucocytes, and infected red cells. A pronounced condition of œdema was therefore nearly always present, and a few interstitial hæmorrhages, with necrosis of cells were found.

The deposit of pigment was usually more abundant in the spleen than in any other situation, and in acute cases was uniformly distributed in the macrophages, endothelial cells, and leucocytes of the pulp, while the Malpighian bodies were almost invariably free (Plate XIV, Fig. 16).

In the acute cases, the distribution of pigment was very uniform throughout the pulp. In a case of fatal typhoid fever which had been free from malarial paroxysms for three weeks, the pigment had been gathered in a network, with rather coarse meshes, throughout the pulp (Plate XIV, Fig. 17); while in another case of chronic malaria three months after the last acute seizure, the pulp was nearly free from pigment, which was gathered in large black intra- or extra-cellular blocks in the septa and walls of arterioles, and about the follicles (Plate XV, Fig. 18).

MARROW.

A chocolate tinge of the marrow expressed from ribs and vertebræ was a characteristic change observed in the acute cases, and, in general, this change in the marrow kept pace with the similar alteration of the spleen. In Cases I and II there was chocolate-colored marrow in the middle third of the humerus and clavicle, indicating an extensive increase in the natural limits of red marrow. Bignami demonstrated such a hyperplasia throughout the femur.

Of the changes in the marrow referable to the growth of parasites,

cellular hyperplasia, obstruction to the circulation, and deposit of pigment, it may be said that they are very similar to and of equal extent with those of the spleen. In Case IV there was an excessive accumulation of parasites in the marrow, the majority of red cells being infected with one or more rings, and multiple infection being very frequent. No infected nucleated red cells could be found.

Usually the number of parasites demonstrable in smears was moderate, though larger, as a rule, than in the liver or spleen. Crescents were not abundant in the tissues of any of the fatal cases, and when present they were not seen in unusual numbers in the marrow, as found by Councilman,[16] Marchiafava, Bastianelli and Bignami ('94), nor in the spleen, as stated by Bignami. In Case I, in which enormous numbers of crescents were found in the blood for two weeks, largely disappearing before death, there were very few crescents to be found in the marrow smears, and the pigment deposits in the marrow were comparatively scanty. This condition is not entirely in accord with the observations of the authors mentioned. In one case (VII) there were numerous small capillary hæmorrhages in the marrow.

Relation of lesions in marrow to malarial anæmia.—The chief interest in the lesions of the marrow in malaria lies in their relation to malarial anæmia, and in this field the studies of Bignami and Dionisi[17] are most complete. The changes in the blood and marrow in the writer's cases accord in a considerable degree with the classification given by these observers.

The most striking of the series in this regard is Case IV, in which there were the lesions of pernicious anæmia in the blood and marrow, and an excessive accumulation of parasites in the marrow. Besides the changes referable to the presence of many parasites and much pigment, the marrow showed well-marked cellular hyperplasia, leading to atrophy and disappearance of fat cells, which are normally present in the marrow of the vertebral bodies. In sections, this hyperplasia seemed to affect principally the large and small mononuclear cells, while in smears the new cells could be divided among the neutrophile myelocytes and the lymphocytes. These cells, together with macrophages and swollen endothelial cells, appeared to cause more than the usual obstruction to the circulation of the marrow, yet no necroses were discovered. The islands of nucleated red cells commonly seen in normal marrow were, in this case, entirely lacking in sections and smears.

[16] *Amer. Journ. Med. Sciences*, 1885, lxxxix, p. 416.

[17] Atti d. XI. Congr. med. internaz., Roma, 1894, ii, p. 235.

In their place there was a considerable number of megaloblasts with increased Hb. A peculiar abnormality, first noted in sections and fully verified in the smears, was the superabundance of giant cells, which occurred in groups of eight or ten. Bignami and Dionisi found this peculiarity in their fourth type of anæmia as occurring in malarial cachexia, and which was marked also by a condition termed by them sclerosis of the marrow. No changes to which the term sclerosis could be applied were found in the marrow in any of the present cases. The examination of the blood, showing the presence of a majority of megalocytes with increased Hb., and of many megaloblasts, together with the condition demonstrated by smears and sections of the marrow, indicates that the fetal type of blood formation had been established in this case, and warrants its classification as pernicious anæmia.

That such changes in the marrow are rather frequently initiated by malarial infection there can be no doubt, as shown by the pathological studies of Bignami and others. In my series of blood-examinations in malaria at Montauk and elsewhere there are no less than 19 cases in which pronounced features of primary pernicious anæmia were observed.

Some regard such evidence as demonstrating the lack of specific quality in the changes commonly regarded as pathognomonic of primary pernicious anæmia. Bignami, however, believes that these cases are still to be regarded as true examples of primary pernicious anæmia, claiming that the reversion of the marrow to the embryonal type of blood-formation, as seen in some cases of malarial anæmia, is not referable exclusively to the infection, but partly results from other associated causes not definitely understood. With this view I am in accord. The majority of cases of pernicious malaria develop the changes of secondary chlorotic or pernicious anæmia, but some show those of the primary pernicious anæmia. The essential difference is not in the cause but in the character of the anatomical changes in the bone marrow. In each instance the changes are initiated by malarial infection. In one case they are maintained almost exclusively by that infection, with which they are more or less proportionate, but in the other they are maintained by the peculiar changes in the marrow which when once initiated may progress independently. This view of the pathology of malarial anæmia, instead of weakening the evidence in favor of the specific nature of the changes in primary pernicious anæmia, furnishes, on the contrary, very strong proof of the specific quality of this condition of the blood.

The lesions found in the present cases, and in some others not reported in this series, indicate that the changes in the marrow in fatal cases of acute malaria follow one of two types:

(1) The cellular hyperplasia is pronounced, the nucleated red cells are abundant and tend to increase in size, the eosinophile cells, giant cells, and lymphocytes are over-abundant, while the fat cells are compressed and atrophic. With these changes, the blood shows moderate or severe anæmia of the chlorotic type, with a marked tendency to develop the signs of pernicious anæmia, which not infrequently become distinct. Pigment deposits and parasites are often unusually abundant as in Cases II and VI.

(2) The cellular hyperplasia is moderate, fat cells being abundant in the vertebræ and persisting in the ribs. Nucleated red blood cells, eosinophile cells, and giant-cells are deficient. The blood shows severe anæmia of chlorotic type. There may be considerable differences in the size of the red cells, but the Hb. is very deficient. The leucocytes are usually diminished, and eosinophile cells are scarce. Pigment deposits and parasites are usually not very abundant (Cases III and VI).

In attempting to draw these general distinctions it must be admitted that the present observations are too limited to fully establish general rules.

The natural limits of red marrow in the normal subject are rather variable, and the quantity of fat in the ribs and vertebræ decidedly so. Moreover, in a series of routine cases coming to autopsy from various causes I have found great dissimilarity in the appearance of the marrow cords, and of smears therefrom, which it is difficult to connect with the various states of nutrition and disease in the subjects. There will be no harm in describing the above types of changes in the marrow of pernicious malaria, if it is understood that it presents a tentative classification which is to be readjusted on the evidence of future investigations.

LEUCOCYTOSIS OF MALARIA.

Most observers have found very little change in the number of leucocytes in the finger blood during acute malarial attacks of average severity. This absence of leucocytosis with a rapidly rising temperature may be found of considerable corroborative value in the diagnosis of malarial fever.

A slight leucocytosis at the beginning of the paroxysm has been noted by Kelsch, Billings,[18] Vincent,[19] and others, but the numbers usually remain

[18] *Bulletin of the Johns Hopkins Hospital*, 1894, v, p. 89.

[19] *Annales de l'Institut Pasteur*, 1897, xi, p. 891.

below 10,000, while the percentage of polynuclear cells is increased. Vincent finds that quinine tends to increase the polynuclear leucocytes during the entire paroxysm. With the falling temperature and during apyrexia the leucocytes are usually distinctly diminished (2000-4000), especially the polynuclear forms, giving a relative lymphocytosis.

Except during the 3 to 4 hours immediately following the chill, therefore, malarial blood usually shows a diminished number of leucocytes, and a distinct relative lymphocytosis. The lymphocytes, small and large, may sometimes become quite numerous, especially in well-established cases. This fact accords with the increased cellular activity of the lymphoid tissues shown by microscopic examination of the viscera.

Bastianelli refers the loss of polynuclear leucocytes to the increased phagocytic activity of these cells. Vincent noted a periodical decrease in the number of large mononuclear cells, which he refered to the same process.

In the severer æstivo-autumnal paroxysms many observers have noted a distinct leucocytosis. Kelsch found that the leucocytosis of pernicious malarial attacks often consists in marked lymphocytosis. Bastianelli and Bignami find that in addition to various inflammatory complications, leucocytosis in pernicious malaria may result from rapidly progressive anæmia. They find it to be of frequent occurrence in hæmoglobinuric fever, and in cases attended with severe diarrhœa.

The presence of eosinophile cells may be noted in most cases of malarial fever, and these cells are usually increased in number during afebrile periods. Grawitz rightly regards this feature as of diagnostic importance, as in most diseases likely to be confused with malaria eosinophile cells are long absent or scarce. Bastianelli and Bignami found that eosinophile cells diminish during the paroxysm, and increase during apyrexia, while the blood is regenerating. In two cases of pernicious malaria, with many parasites, they found many mononuclear leucocytes and a very few eosinophile myelocytes, similar to those seen in myelogenous leukemia.

In the present cases the usual behavior of the leucocytes was noted in the majority of instances.

In some severe and prolonged cases the lymphocytosis was a marked feature, these cells being distinctly increased in number. Eosinophile cells were never found greatly increased, but their nearly constant appearance in patients who were suffering from continuous or intermittent fever furnished a somewhat peculiar feature of the blood of malarial infection.

In some pernicious cases moderate polynuclear leucocytosis was observed; and in a few cases complicated by pneumonia, colitis, or severe cachexia, a considerable leucocytosis was found. In two of the fatal cases reported in this study, and in others, ante-mortem leucocytosis was observed, but the majority of fatal cases failed to show distinct leucocytosis. These estimates were all based on the examination of dry specimens.

Pigmented leucocytes were seen in the majority of cases, most abundantly in the severe and long-established fevers. They were found in nearly all fatal cases, but were most abundant in a case which recovered (No. 238). It appeared that pigmented leucocytes were more closely related to the severity of the antecedent paroxysms than to the extent of the pigment deposits in the viscera.

They were most abundant during and shortly after the febrile period, but were repeatedly found in afebrile cases and after parasites had disappeared from the blood. The phagocytic cells seen in the blood included mononuclear and polynuclear leucocytes and endothelial cells. The large and small mononuclear cells were most often found to contain pigment or parasites (Plate X, Fig. 6), but in a few cases, for reasons not clear, large numbers of polynuclear leucocytes were found harboring rosettes, other forms of parasites, and pigment (Plate X, Fig. 5). In a few cases very large endothelial macrophages were found in the blood containing parasites in all stages of degeneration (Plate X, Fig. 4).

The objects englobed by phagocytes as seen in the circulation included: (1) Parasites, free or inclosed in red cells; (2) Pigment elaborated by parasites, usually in small clumps, sometimes in large masses; (3) Hæmatoidin derived from the destruction of red cells; (4) Hæmosiderin derived from the detritus of red cells; (5) Intact or broken red cells; (6) Other leucocytes. Crystalline pigment was often seen in leucocytes in sections of tissues but never in the circulating blood during life. The degenerative changes in phagocytic leucocytes mentioned by Bastianelli and Bignami, including vacuolation and diminished staining capacity of nuclei, were noted in many severe cases. The number of vacuolated leucocytes (Plate XI, Fig. 8) found in the blood was always considerable and sometimes very large.

LYMPH NODES.

The abdominal lymph nodes were examined in all cases, and usually found moderately swollen, but always without signs of pigmentation. Microscopic examination was made in two cases only; in these the nodes were hyperplastic, the lymph sinuses were invisible, the stroma contained many mast-cells, and there was a scanty deposit of pigment, mostly in the endothelial cells.

LUNGS.

In gross appearance the lungs in pernicious malaria, both in these and in other reported cases, presented little that is characteristic of

the disease. They commonly contain a considerable deposit of pigment and are often very dark colored, but this feature is usually not distinctly characteristic, owing to the simultaneous presence of anthracotic pigment, hypostatic congestion, and frequently of jaundice.

Somewhat peculiar areas of lobular pneumonia were found in two cases, and have been described by Bignami, but the exudate was composed of the ordinary elements, and the inflammation was not specially connected with the growth of parasites.

Microscopically, the lungs are usually found to contain a very large number of pigmented cells, sifted from the general circulation, and in some cases a moderate additional deposit elaborated by the parasites in the pulmonary capillaries. Some large pulmonary macrophages, probably derived from other viscera, were found, filling a considerable length of the capillary. Thrombi composed of such cells, with pigmented leucocytes, swollen endothelium, and infected red cells were not infrequently found in the pulmonary capillaries of Case II. The lungs were œdematous but free from hepatization.

There appear to be no recorded instances of an excessive growth of parasites in the lungs comparable with that found in other viscera. Bignami speaks of a rusty color of the sputum in some cases of bronchitis in pernicious malaria, which he regards as of little clinical import, and I can find no report of the microscopic examination of such sputum, or of the lungs and bronchi in such cases, to show that an excessive growth of parasites in the lungs may give this character to the sputum.

From what has been shown of the action of the lungs in sifting bacteria and leucocytes from the blood in infectious diseases, it might be expected that the lungs would suffer severely in malarial infection. Their comparative immunity in this instance may perhaps be referred to the rapidity of the capillary circulation and the abundance of oxygen.

CARDIAC MUSCLE.

French writers especially (quoted by Laveran) have laid emphasis on the pallor and flaccidity of the myocardium in pernicious malaria. That condition was noted in nearly all the present cases at autopsy. In Case II nearly all tissues, including the heart muscle, were more or less discolored by malarial pigment. In Case VII, the heart muscle was generally light colored, but exhibited a slight brownish tinge referred at the time to the jaundice.

Microscopically, there were no pronounced changes found in the

muscle cells, but the perinuclear mass of large greenish granules was sometimes very abundant. Mast-cells were sometimes found in unusual numbers in the endomysium. Distinct fatty changes were not observed. With one exception, little pigment and few parasites were found in the capillaries. In Case II there was a notable exception to the usual rule and very large numbers of young parasites and pigmented cells were found completely filling distended capillaries throughout the heart wall (Plate X, Fig. 7). Although numerous parasites were found in other viscera and the brain was not examined, there was an excessive proportion in the heart muscle, while cerebral symptoms were late and cardiac failure was the most prominent clinical symptom.

Of similar cases, Benvenuti [20] has reported one in which the heart's capillaries were filled with infected red cells, the endothelium pigmented and degenerated, the muscle fibres swollen, their striation indistinct and the yellow pigment increased. In the brain it appears that there were as many parasites as in the heart, and many were also found in the kidney. Coma and cardiac dyspnœa were the principal symptoms. In another case Benvenuti found many parasites in brain, kidney, and heart, while the principal symptom was stupor. The clinical histories accompanying these reports are meagre for the present purpose, and it is difficult to judge of the relative number of parasites in the different viscera. I have been unable to find in the literature any other reports of such cases. Theobald Smith, however, finds that in Texas cattle fever very large numbers of parasites are commonly found in smears made from the heart muscle.

The available evidence, therefore, hardly seems to warrant a positive conclusion that acute cardiac failure in pernicious malaria may result from a massing of parasites in the cardiac muscle, and it would be safer to conclude from the present case merely that the condition of the cardiac and skeletal muscles demands more attention than is generally paid to these tissues in pathological studies of malaria.

KIDNEY.

Grossly, the kidneys in pernicious malaria usually give evidence of acute degeneration in their slightly increased size, diminished consistence, rather pale cortex, and indistinct but regular markings. There are usually no characteristic signs of malarial infection. In Case I the medulla and papillæ exhibited a somewhat peculiar darkening of color, from the unusual deposits of pigment. In some cases of black-

[20] *Policlinico*, 1896, iii-M., p. 390.

water fever the presence of extra-vascular blood in cortex and medulla considerably alters the above description, as in Case VII. In Case V the cortex was very light colored, while the medulla and papillæ were deep rust-colored from the large numbers of parasites in the capillaries in these areas. In this case there was also a large superficial anæmic infarct.

Microscopically, the usual lesions, well illustrated in the present cases, consist in granular, hydropic, and fatty degeneration, pigmentation by hæmosiderin granules, and sometimes isolated or diffuse necrosis of convoluted-tubule cells. The intertubular capillaries usually contain moderate numbers of pigmented leucocytes, macrophages, and infected red cells, while the glomeruli gather a larger number of similar elements.

The kidney rarely suffers from the accumulation of growing parasites, as does the brain, the mucous membranes, etc., a fact referred by Bignami to the rapid circulation in the organ. In blackwater fever I can find no reports of excessive numbers of parasites in the kidney, and in other cases, as a rule, the numbers of parasites have not exceeded those in the peripheral blood.

On the other hand, the eliminative function of the kidney exposes it to the effects of the toxæmia of malaria, so that albuminous urine is a very common clinical sign in acute pernicious malaria, especially if protracted, while the condition of the renal cells in the present cases, and in most others reported, shows nearly constantly a considerable damage to the organ from this cause. The lesions, however, were, in most of the present cases, of a purely degenerative type, without evidence of exudation into the stroma or other changes in the connective tissue. In Case III the degenerative changes were very intense, many cells were extensively eroded, and some necrotic, and although the tubules were dilated, and casts and granular coagulum were present in considerable abundance, indicating a near approach to exudative nephritis, yet there were no leucocytes in the tubules and no leucocytes or serum in the stroma.

With the minor exception of a slight glomerulitis, described in one case by Bignami, no more serious lesions have been found in the kidney in uncomplicated acute malaria. That the lesions are entirely out of proportion to the number of parasites in the organ and are probably of toxic origin has been generally accepted. The peculiar very abundant deposit of hæmosiderin granules in the renal cells, as illustrated in extreme degree in Cases III and VII, is a somewhat characteristic feature of the malarial kidney, but is sometimes seen in other diseases.

The anomalous condition of the medullary tubules in Case I, the lining cells containing enormous numbers of pigment wreaths, has already been considered in the report of that case (p. 124). Changes characteristic of "blackwater fever" were found in Case VII, and consisted in extreme congestion, numerous small hæmorrhages, excessive deposits of granular and crystalline pigment, probably derived from dissolved hæmoglobin, and peculiar necrosis of tubule-cells. In Case V the intense and peculiar degeneration of the tubule cells seems referable in part to the obstructed circulation from thrombi of infected red cells, while the hæmorrhagic type of the nephritis was distinctly the result of localization of parasites in the renal capillaries.

GASTRO-INTESTINAL TRACT.

In six of the present cases no gross lesions in the gastro-intestinal mucosa were to be found. In one case (III) considerable diarrhœa of long standing failed to leave any traces of inflammation in the intestine or colon. In Case VII an intense catarrhal colitis was apparently not caused directly by the malarial infection, as only moderate traces of parasites and pigment were found in the intestinal wall.

In Case VIII amœbic dysentery of moderate extent was combined with intense general malarial infection, but sections of the ulcers, while showing large numbers of the amœbæ, exhibited very scanty traces of malarial infection, no parasites and few pigmented leucocytes being found.

These cases indicate that marked diarrhœa in pernicious malaria may occur without anatomical changes in the intestinal mucosa, or may result from a secondary catarrhal colitis not directly caused by the malarial parasite, or may result from amœbic colitis, in which the malarial infection is not directly concerned. It has already been shown also that hæmatemesis, severe diarrhœa, and paroxysms resembling Asiatic cholera occur as the result of the massing of parasites in the gastro-intestinal mucosa.

According to Marchiafava, in the choleriform malarial cases, the parasites are very abundant in the mucosa of the small intestine, but scarce or absent elsewhere. Infected red cells may be identified in the stools, which are often bloody. The intestine contains bloody fluid and flocculi of mucus. The mucosa is swollen, congested, and shows superficial hæmorrhages and erosions. It is often dark brown or chocolate colored, while the unaffected light solitary follicles project prominently. Microscopic examination shows: (1) injection of vessels of mucosa and especially of villi with blood containing many parasites; (2) necrosis of epithelium of villi and mucosa, in which areas neither nuclei nor parasites can be stained; (3) infiltration with

leucocytes beneath the necrotic areas; (4) bacterial invasion of necrotic tissue; (5) mitotic division of nuclei of sound epithelial cells; (6) freedom from parasites of vessels of submucosa, which contain many pigmented leucocytes.

In Case II. although a considerable number of parasites was found in the intestinal mucosa, there were no changes referable to their presence, and the patient did not suffer from diarrhœa.

There has apparently been little opportunity to ascertain the character of reparative processes which may follow the acute lesions in choleriform pernicious malaria, since such cases are usually fatal. Pensuti,[21] however, has furnished one observation of interest in this connection. A patient who had suffered in November, 1892, from severe malaria with vomiting and profuse diarrhœa, was treated actively by quinine, but the diarrhœa persisted and he died of broncho-pneumonia in February, 1893. In the intestine, especially in the ileum, the mucosa was hyperæmic, and showed some amyloid changes in the vessels. In many places there was complete disappearance of the glandular layer, which was replaced by young connective tissue. The remaining islands of glandular tissue showed marked hypertrophy of alveoli. A good deal of pigment (character not stated) was found in the mucosa. Pensuti regarded the lesion as referable to the toxic effects of malaria.

There seems to be little pathological evidence on which to discuss the relation to malaria of some forms of tropical colitis not amœbic, but often associated with pernicious malaria.

CENTRAL NERVOUS SYSTEM.

Gross appearances.—In some cases of comatose malaria with rich infection the brain presents a characteristic brownish discoloration, most marked in the gray matter, which results from deposits of pigment. This condition has been found in a considerable number of cases, but by no means in all. In 40 cases Kelsch and Kiener found marked discoloration of the cortex in 9, and faint changes in 11 cases. When present it invariably indicates the presence of a very large number of parasites and much pigment in the brain tissue. Its absence, on the other hand, by no means excludes the presence of a large number of pigment-free parasites in the gray matter, a fact which was apparent in some of my cases not reported here, and which is referable to several causes. The æstivo-autumnal parasite is not always a very active pigment-producer, and Guarnieri and Bignami have depicted cerebral capillaries completely thrombosed by pigment-free rosettes. Very large

[21] *Gazz. med. di Roma*, 1893, xix, p. 121

numbers of young parasites may therefore be present in a tissue which shows very little gross evidence of pigmentation. The discoloration resulting from jaundice, which frequently complicates fatal malaria, may obscure the effects of malarial pigmentation. Finally, as will subsequently be shown, the majority of cases of comatose malaria do not exhibit a massing of parasites and pigment in the brain, so that the characteristic discoloration of the gray matter sometimes found is not to be expected in these cases. Accordingly of eight cases of comatose pernicious malaria in which I was able to examine the brain a brownish discoloration, which could be regarded as absolutely characteristic of malaria, was not found in any. Most of the cases were jaundiced, but in two others showing many parasites the discoloration present was too faint to be regarded as pathognomonic of the disease.

Multiple hæmorrhages in the gray matter have been described in comatose malaria by Guarnieri, Bignami, Marchoux,[22] Monti, Bastianelli, Blanc, and Spiller.[23] These lesions were not discovered in any of my cases. In Bastianelli's case the hæmorrhages were limited to the cerebellum, while disturbances of equilibrium were said to have been prominent symptoms during life. In a case reported by Blanc, in addition to numerous capillary hæmorrhages, there was a large subcortical clot.

Pial œdema of moderate grade has been found in the majority of cases, but cannot be regarded as of special significance.

Blanc and Borrut claim to have observed true inflammatory exudative lesions in the brain in pernicious malaria, but their observations have not been confirmed. Maillot, in 1851, mentioned two cases of red softening of the lower dorsal cord.

Microscopic changes.—The microscopic appearances of the brain tissue in the typical cases of pernicious malaria of cerebral type are too well known to warrant minute description here. The principal feature is the massing of red cells infected with various forms of æstivo-autumnal parasites in the capillaries. Usually the parasites have been found uniformly distributed in the brain and cord, but Marchiafava [24] observed a case with bulbar symptoms, in which there was special localization in the medulla, and in a case of Bastianelli's the limitation of hæmorrhages to the cerebellum indicated a special massing of parasites in that region. The numbers of these parasites are sometimes

[22] *Annales de l'Institut Pasteur*, 1897, xi, p. 640.

[23] *Amer. Journ. Med. Sciences*, 1900, cxx, p. 629.

[24] *Lavori d. III. Congr. di med. int.*, Roma, 1890, p. 142.

enormous, often partly or completely occluding the lumen of the vessel. Complete thrombosis frequently results from the agglutination of infected red cells, pigmented leucocytes, and swollen endothelial cells. Capillary hæmorrhages result in the neighborhood of such thrombi, and are probably preceded by degenerative changes in the capillary endothelium. While most of the fixed pigment is found in the endothelium and in circumvascular lymph spaces, parasites are rarely seen in the endothelial cells. Monti, however, describes well preserved parasites in degenerating endothelia and believes that the parasites are sometimes capable of development in these cells. Such an occurrence is at least unusual.

To the general condition of obstructed circulation it is probably safe to refer the marked cerebral symptoms of such cases of acute pernicious malaria.

The infiltration of pericellular lymph spaces with small round cells, which is seen in many infectious diseases, was noted in some of the present cases, but not in excessive degree.

The deposit about the ganglion cells of peculiar masses of variously twisted threads and rods staining densely with methylene blue, was noted in the description of Case II.

Considerable attention has been paid in recent years to the condition of the *ganglion cells* in comatose cases of malaria. Monti studied the changes in the ganglion cells in several cases by means of Golgi's method. In some instances no important alterations of the ganglion cells were found. In others, with severe nervous symptoms, extensive changes were discovered, of focal distribution, affecting principally the dendrites. These processes were thinned in places and beset with many small swellings, and the changes affected either the finest twigs only, or the entire dendritic system, or the cell body itself was shrunken and irregular. Some dendrites showed the usual changes of varicose atrophy. The axis cylinder processes were sometimes found normal, but in the severe comatose cases extensive changes of the above types affected the axis cylinder as well as the dendritic processes. Monti referred the changes to occlusion of capillaries, finding them very similar to the lesions produced by multiple emboli produced by intravascular injection of lycopodium.

The lesions in the ganglion cells demonstrated by Nissl's method have appeared in the present cases not to differ essentially from those seen in other infectious diseases with marked cerebral symptoms. These changes consist principally in varying degrees of chromatolysis

affecting the cortical cells rather uniformly, but the cells of the bulbar nuclei more irregularly. The earlier stages of the lesions include reduction in size, irregularity and subdivision of the chromatic bodies, usually beginning in the dendrites, later involving the cell body. In more advanced stages the chromatic bodies are largely destroyed, and a moderate number of cells may be entirely bereft of chromatic substance. The more serious lesions of true acute degeneration, such as destruction of cyto-reticulum, shrinkage and cleavage of cell body, vacuolation, and nuclear changes, were seldom seen in my cases.

As to causation of these lesions, probably local disturbances of circulation are a more important factor in malaria than in most other diseases, but the comparative uniformity of the lesions noted indicates that a general toxæmia is, even in cerebral cases, the more important pathogenic agent.

A great variety of *nervous symptoms* referable to disturbances of the central nervous system have been attributed to malarial infection by early writers whose reports lack the evidence, now demanded, of a positive blood examination. The rather extensive literature of this subject has been fully considered by Mannaberg, and in a recent article by Bardellini.[25]

Since the discovery of the parasite, there is good clinical and in some cases anatomical evidence indicating that many of the nervous symptoms early referred to malaria may really be dependent on mechanical or toxic lesions resulting from this infection. Authentic cases have been reported showing hemiplegia (Marchiafava, Vespa); general convulsions (Marchiafava, Bignami, Baccelli); tetanic spasms after acute malaria (De Francesco); athetoid movements (Boinet and Salebert); disturbances of equilibrium (Bastianelli, Bignami); trismus, nystagmus, various toxic and clonic spasms (by many writers); post-malarial psychical disturbances (Pasmanik, Ségard, and many others); symptoms of disseminated sclerosis, paresis, increased reflexes, ataxia, bulbar paresis, in two cases (Angelini and Torti); electric chorea of Dubini (Bastianelli and Bignami); paralysis of the bladder from spinal lesions (Bardellini); hyperidrosis from affection of the sympathetic (Bardellini); and polyneuritis somewhat resembling Landry's paralysis (Bardellini, Torti, Mesnard). Bardellini concludes that when nervous lesions are transitory they are probably embolic, but when permanent they are probably complicated by multiple hæmorrhages. In a review of the recent literature he could find no satisfactory evidence that periodic neuralgias may be directly referable to malarial infection.

It must be admitted that while the etiology in most of the above cases was probably malarial, yet the evidence is generally unsatisfactory and, usually lacking anatomical support, is inconclusive, while in some instances the malarial infection was clearly secondary and had nothing to do with the chronic lesions.

[25] *Annali di medicina navale*, 1898, iv, p. 919.

Coma in pernicious malaria.—From the study of 64 cases of malarial coma at Montauk and in New York, some of which are included in the present series, it appears that this cerebral symptom in malaria occurs in three rather distinct clinical pictures and under three entirely different pathological conditions.

(1) *Malarial coma may be referable to massing of young amaboid parasites in the cerebral capillaries.*

This type of coma, which has long been recognized as a frequent form of pernicious malaria, is illustrated in Case III in which the cerebral symptoms were found to be associated with an extensive massing of parasites in the cerebral capillaries, while the deepening stages of coma could apparently be connected with the increase in size of the parasites and the gradual filling of the vessels with thrombi of infected red cells, pigmented leucocytes, and swollen endothelial cells. No capillary hæmorrhages were discovered.

Clinically, the coma resulting from this pathological condition is rather slowly established in the course of active infections, when many young parasites are found in the finger blood and when the temperature is elevated. The patient is usually first delirious, then mildly comatose, then deeply comatose, finally stuporous, with abolition of pupillary and other reflexes, and almost always dies within 48 hours after the beginning of marked cerebral symptoms. Of 11 such cases observed at Montauk 10 died, and very vigorous treatment succeeded in saving only one.

(2) *Malarial coma may be referable to embolic processes with temporary occlusion of vessels in small areas of the brain, and without uniform massing of parasites in cerebral capillaries.*

In these cases the coma develops suddenly and may be as suddenly recovered from. In a case previously reported the patient three times in five days fell back unconscious in bed, his pipe dropping from his mouth, but after a variable period he recovered consciousness, picked up his pipe and resumed smoking. From this very transient form the duration of the coma may be much more prolonged and serious, but it is seldom fatal. It may occur in febrile or afebrile cases and may exhibit distinct symptoms of focal irritation or meningitis. In the blood, few or many crescents, sometimes tertian parasites, but very few rings are usually found, and occasionally no parasites can be discovered. Emboli of parasites, pigmented leucocytes, and visceral macrophages, seems to be the only anatomical lesion which can explain such symptoms. They arise in established cases of the disease and

on microscopic examination extensive malarial lesions are found in the viscera but few or no parasites are to be found in the brain. Although crescents or tertian parasites may be abundant in the peripheral blood in these cases, I have not seen, nor been able to find in the literature report of any case in which large numbers of crescents or tertian parasites were found occluding cerebral vessels, and it appears that these parasites do not exhibit the tendency to unequal distribution in any degree comparable with the fertile æstivo-autumnal forms.

(3) *Malarial coma may be referable to the general toxæmia of the infection.*

In these cases the coma usually develops slowly but may in cachectic cases be ushered in suddenly, apparently by some embolic process. It is often of prolonged duration and not being caused by massing of young parasites in cerebral vessels it is unaffected by quinine. Occurring only in severe cases and being associated with serious toxic lesions in many viscera it is nearly always fatal.

Cases I and IV of the present series illustrate this type of coma. These patients were comatose, one at least three days and the other for two weeks before death. As no other cause for the coma was found it had to be referred to the malarial infection, which was very severe and long established. These cases differed radically from the classical type of comatose malaria, as in one only a few crescents, and in the other only tertian parasites, were present in the blood, and no parasites and comparatively little pigment were found in the brains. They show conclusively that the coma of pernicious malaria is not always referable to the presence of parasites in the cerebral capillaries. In the Montauk series[26] there were four other fatal cases of comatose æstivo-autumnal malaria in which crescents only were found in the blood, and one other fatal tertian case with prolonged coma. Jancsó and Rosenberger[27] also have reported a fatal comatose case in which the brain contained few parasites, which were abundant in the other viscera, the coma being referred by the authors to a toxic origin. It is possible that too little attention has been paid to the opinion of Guarnieri that malarial coma may sometimes be caused by obstruction to the portal circulation. This opinion was based upon the evident obstruction to the hepatic circulation commonly found in the liver of pernicious malaria, and upon the experimental production in dogs of coma without convulsions by ligature of the portal vein.

[26] Ewing, *N. Y. Med. Journ.*, 1899, lxix, pp. 114; 149.

[27] *Deutsches Arch. f. klin. Med.*, 1896, lvii, p. 449.

Uræmia may possibly be held partly responsible for some cases of this type but the clinical picture is not that of uræmia and the renal changes are not such as are commonly associated with uræmia.

Distinct differences in prognosis belong to these three varieties of coma.

Of eleven cases in which coma supervened during the development of a numerous brood of parasites, ten were fatal. Some of these, but not all, were of the classical cerebral type with massing of parasites in the brain. The energetic use of quinine has some influence in such cases.

When coma develops gradually in severe and long established cases, with few young parasites in the blood, it is usually of toxic origin, is unaffected by quinine, and is almost invariably fatal.

Of 33 cases of coma developing, often suddenly, in cases with crescents only in the blood, there were but three fatalities.

THE MALARIAL PIGMENTS.

Two forms of malarial pigment have long been recognized. One of these, melanin, is granular, brownish, elaborated directly by the parasite, according to Sacharoff from the nuclear remnants in the red cell, and fails to give the Prussian blue reaction of hæmosiderin. It is not, on that account, necessarily free from iron. This pigment is dissolved by ammonium sulphide and readily by heat, but long resists the action of strong acids and alkalis, and I have found it to be insoluble in hardened tissues by chloroform, ether, or carbon bisulphide.

The other described form of malarial pigment occurs more abundantly in protracted cases, as small yellowish granules, located principally in the tissue cells, especially in the liver, kidney, spleen, and marrow. It yields, when fresh, the reaction of Prussian blue, but gradually loses this reaction. It is probably the hydrated oxide of iron (Thoma).

When the Hb. of red cells is dissolved in the plasma, as occurs in poisoning by potassium chlorate, arsenic, etc., and in diseases such as pernicious anæmia, scorbutic ailments, malaria, septicæmia, etc., it may be found in the tissues in granular or crystalline form and of dark brown or reddish color. Sometimes such Hb. granules are soluble in water, but more often they are altered in some way and become insoluble in water, when they have been called "parahæmoglobin" by Zenecki. Parahæmoglobin is very nearly identical with hæmatoidin, which is frequently precipitated in granular or crystalline form from blood extra-

vasations, and both are soluble in chloroform and carbon bisulphide. Perls, Thoma, and Ziegler, on whose authority these statements are made, leave one to infer that since melanin, the black malarial pigment, is insoluble in chloroform, etc., this test furnishes a chemical reaction which may distinguish hæmatoidin from malarial pigment. On submitting formalin-hardened tissues containing old blood extravasations to the action of chloroform, I find that two weeks' exposure has no effect upon the granules and crystals of hæmatoidin. Possibly they had been altered by age, or by the action of formalin, but the same result was obtained in tissues hardened in alcohol. Both hæmatoidin and black malarial pigment were dissolved by ammonium sulphide. Accordingly I failed to find the described chemical reactions of hæmatoidin of practical value, at least in hardened tissues, in distinguishing between malarial pigment and the granular and crystalline deposits derived from destruction of red blood cells.

Another difficulty arises in the identification of malarial pigment in tissues. Many cases of pernicious malaria are attended with marked jaundice. In many cases of jaundice from other causes bilirubin, now regarded as identical with hæmatoidin, is deposited in granular or more often in crystalline form, and is identical in color with hæmatoidin and with much pigment found in the bodies of malarial parasites. In sections of two cases of marked jaundice following pneumonia, one in a young infant, the other in an adult, I found many crystals and granules of bilirubin (?) (Perls, Ziegler) in the hepatic endothelium. In the case of the infant, they were found in large masses in the liver cells also, as well as in many leucocytes in various organs, which very closely resembled the pigmented leucocytes of malaria. Now bilirubin in fresh bile is turned green by formalin and gives Gmelin's reaction, but the crystals in neither of these cases turned green in formalin nor gave Gmelin's reaction. There were, however, other greenish particles in the formalin-hardened sections of both livers. In both cases chloroform failed to alter the crystals in two weeks, and they failed to give the Prussian blue reaction. That the crystalline deposits in the liver cells of the new-born infant were derived from the bile there can be little doubt, and the failure of the reactions may be explained, as is done by Gerhardt and others (quoted by Perls), who find that in many forms of jaundice urobilin and not bilirubin is formed. Urobilin fails to give Gmelin's reaction. Formalin-hardened sections of these tissues were treated for 24 hours, also, in carbon bisulphide, and in ether, but no change in the pigment was observed. The same negative result was obtained with sections of malarial tissues.

These cases are briefly referred to in order to point out that the jaundice of infectious diseases may cause deposits of pigment which are indistinguishable morphologically, and by all ordinary chemical procedures, from much malarial pigment. Various reports of the finding of malarial pigment in the liver cells in pernicious malaria have possibly not been made with full recognition of this fact. The positive identification of malarial pigment therefore becomes a matter of great difficulty, for it appears from the above considerations that in malarial fever one may meet with granular, sometimes crystalline pigment particles, free in the vessels or englobed in various cells, not giving the Prussian blue reaction, nor dissolving in chloroform, ether, or carbon bisulphide, but dissolving in ammonium sulphide, which may have any one of the following origins:

(1) Pigment elaborated by the intracellular parasite.

(2) Hæmatoidin derived from the remnants of infected red cells.

(3) Hæmatoidin or altered hæmoglobin deposited in granular or crystalline form from red cells dissolved in the plasma. (Hæmoglobinuric fever, jaundice, extravasated blood, post-mortem processes.)

(4) Bilirubin or urobilin granules or crystals.

Throughout the study of the present cases, in addition to the dark brownish granular pigment inclosed in cells or parasites, larger dark brown particles exhibiting more or less crystalline forms were frequently found. When scanty they were usually limited to the spleen, but in Case VII there were excessive deposits in all viscera except the liver and spleen. Since the pigment in parasites is invariably granular and not crystalline—possibly there are rare exceptions in the crescentic bodies—the above considerations render it extremely probable that the strictly malarial pigment, i. e., that derived from parasites, is never found in crystalline form, and that all such crystals ought to be referred to some other origin. Moreover as the detritus from dissolved red cells may be found as brownish granules in the bodies of phagocytes, it cannot be claimed, even for the pigmented macrophages, that all granular pigment is derived from parasites.

When one follows the changes that occur in degenerating parasites and red cells within a macrophage, there are, however, certain long-retained characters which often serve to distinguish the pigment derived from parasites from that resulting from the destruction of red cells.

In sections of tissues, the pigment within parasites is invariably finely granular, first appearing as one or two fine grains, later as a

larger, more or less irregular clump, while in æstivo-autumnal rosettes the pigment appears in a rather compact spheroidal mass of fine granules. In sections, the pigment is usually less compact than in blood smears, in which it may often be found in a single large block with outlying grains. When these pigmented parasites are englobed in macrophages the body of the parasite rapidly disappears, in 4 to 5 hours according to Marchiafava, leaving a small vacuole about the pigment clump, and usually leaving the pigment undisturbed in arrangement of granules for some days. When the pigment clump is englobed after its discharge from the rosette into the plasma, it appears sometimes to retain its compact arrangement, in which case the englobed mass fails to show a surrounding vacuole. In the leucocytes of the peripheral blood, which appear to absorb most of their pigment from the plasma, it is seldom possible to detect any vacuole surrounding the clump. When a red cell is englobed in a macrophage, the Hb. is frequently reduced to brownish granules skirting the periphery of the space originally occupied by the red cell, or sometimes showing a less regular arrangement of fine granules within a large vacuole.

Finally, when a red cell infected by a pigmented parasite is englobed, the destruction of the red cells may leave a peripheral ring of granules, while the larger compact mass from the parasite occupies a central or peripheral position (Plate X, Figs. 1 and 4).

From a minute study of the appearances of intracellular pigment clumps in sections, and especially in smears of tissues, it is possible to follow degenerating parasites and red cells through all the stages just described, and to distinguish in many instances pigment derived from parasites from that resulting from the destruction of red cells. After a variable time all englobed pigment appears to concentrate in more compact perinuclear masses, and the above features can no longer be identified. Some of the larger masses appear to form by the coalescence of two or more vacuoles.

When one compares the deposits of hæmoglobin which occur in inflamed tissues infiltrated with blood, with deposits of malarial pigment, certain characters are often distinctly apparent which serve to distinguish the one from other. Chief among these is the crystalline form of much of the pigment in the inflammatory deposits. In many such cases the formation of intra- and extra-cellular circles of crystalline pigment may be traced from whole or subdivided red cells. Such features were noted especially in the renal tubules of Case I of the present series (Plate XI, Fig. 9). Sometimes the pigment circle repro-

duces the original size of the intact red cell, usually it is smaller, the red cell having been subdivided before its final alteration, while frequently the crystals are isolated and elongated, or of small size resembling granules. No spheroidal clumps of finely granular pigment like that of the æstivo-autumnal rosette were seen in several inflamed mucous membranes with disintegrating blood, examined for this purpose. In cases of pneumonia, septicæmia, and blood extravasations from various causes, along with the crystalline pigment there was much coarsely granular pigment in leucocytes and endothelial cells, but none or very few of the clumps exhibited the finely granular character of fresh malarial pigment.

Since malarial pigment as seen in the parasite is practically never found in crystalline form, and there seems to be no good reason to assume its transformation into crystals after englobement by phagocytes, crystalline pigment must be referred to other sources, principally the destruction of red cells.

According to Thoma, hæmosiderin probably results when the detritus of red cells is acted upon by living tissue cells, while hæmatoidin is produced in the bulk of a blood extravasation where living phagocytes are absent.

The study of hepatic macrophages in malaria shows, however, that englobed red cells may be reduced to hæmatoidin as well as to hæmosiderin. Not infrequently one finds in the bodies of such macrophages red cells which give the hæmosiderin reaction, but show in addition some black hæmatoidin grains. This fact has also been noted by Barker. The two pigments appear to form, in malaria, under nearly identical conditions, and in the large macrophages it appears certain that the majority of englobed red cells are reduced to hæmatoidin.

Not having found in chloroform, ether, carbon bisulphide, acidified potassium ferrocyanide, or ammonium sulphide, any chemical reagent that will distinguish malarial pigment from parahæmoglobin, hæmatoidin, bilirubin, or urobilin, etc., one must apparently rely upon morphology alone for its identification in hardened specimens.

In pernicious malaria, the formation of pigment by the parasite, the intracellular destruction of red cells, the solution of Hb. in the plasma, occurring most extensively in hæmoglobinuric fever, but seen to some extent probably in all fatal cases, the deposit of bilirubin or urobilin crystals or granules in jaundiced cases, all are processes which are variously intermingled in the disease, and in the study of the tissues it is of prime importance (see Cases I and VII) to distinguish as far as possible between them.

Case X has been reported here in order to illustrate the practical importance of the foregoing observations.

Certain alterations of interest were noted in the deposits of pigment in liver and spleen after subsidence of the infection. Throughout the periods of active infection the pigment was richly and uniformly distributed in all parts of the organs. In a case of typhoid fever in a malarious subject dying from perforation three weeks after the disappearance of parasites under quinine, the pigment in the liver was found in less numerous but larger and more compact clumps within the capillaries, but very little had yet reached the portal canals. In the spleen the beginning concentration of pigment had caused the appearance of a pigment network with large meshes, within which were newly-formed cells free from pigment (Plate XIV, Fig. 17).

In another case, dying three months after subsidence of the infection, the greater concentration of pigment is clearly indicated in the photograph (Plate XV, Fig. 18).

Ferrier,[5] in a case dying one month after cessation of fever, found a moderate amount of pigment in the cells of the pulp cords only. In another case, after a similar period, much pigment in nodular blocks 2-3 times the diameter of the splenic cells was found in the splenic cords. Rather less pigment was found in the arterial walls and peripheries of follicles. In a third case, six weeks after acute symptoms, most of the pigment was found in very large cells in the centres of the pulp cords.

DESCRIPTION OF PLATES X-XV.

Plate X.

Fig. 1. Appearance of hepatic macrophage in Case II. Various stages of destruction of infected and uninfected red cells and of parasites.

Fig. 2. Hepatic endothelial cell in established æstivo-autumnal fever, containing pigment.

Fig. 3. Hepatic macrophage in Case VII, fatal infection with large tertian parasite. The pigment is distributed in fine granules and in blocks.

Fig. 4. Endothelial macrophage of circulating blood, containing rings and masses of pigment and degenerating parasites. Red blood corpuscle at the side.

Fig. 5. Polynuclear leucocyte of circulating blood with englobed rosette and full grown parasite.

Fig. 6. The usual pigmented mononuclear leucocyte of the circulating blood.

Fig. 7. Capillary in muscle of the heart, showing numerous intra-corpuscular parasites. Case II.

Plate XI.

Fig. 8. Appearance when stained of vacuolated, pigmented leucocyte, common in malarial blood.

[5] *Arch. de méd. expér.*, 1897, ix, p. 87.

Fig. 9. Pigmentary deposits in cells of renal tubule, from disintegrating red corpuscles. Case I.

Fig. 10. From section of uterine submucosa of Case X, puerperal septicæmia. Deposits of wreaths of granular and crystalline pigment, resembling malarial pigment, in tissue infiltrated with blood.

PLATE XII.

Fig. 11. Early stages of destruction of red corpuscles with deposit of peripheral rings of hæmatoidin. Pigmented leucocytes. Case VII and others.

Fig. 12. Later stages of destruction of red corpuscles with deposit of crystals of hæmatoidin. Case VII.

PLATE XIII.

Fig. 13. Photograph of section of kidney, Case V. Degeneration of renal epithelium and granular coagulum around glomerulus. × 250.

Fig. 14. Photograph of section of kidney, Case V, showing massing of malarial parasites in renal capillaries. × 250.

Fig. 15. Photograph of section × 1000, same case, showing pigmented parasites in renal capillary.

PLATE XIV.

Fig. 16. Photograph of section of spleen, Case VII, showing distribution of pigment in acute pernicious malaria.

Fig. 17. Photograph showing reticular arrangement of pigment in the spleen, three weeks after subsidence of acute malarial infection.

PLATE XV.

Fig. 18. Photograph showing disposition of pigment in the spleen, three months after acute malarial infection. Case IX.

Fig. 19. Photograph showing distribution of pigment in the liver, three months after acute malarial infection. Case IX.

FIG. 1.

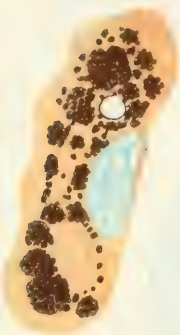
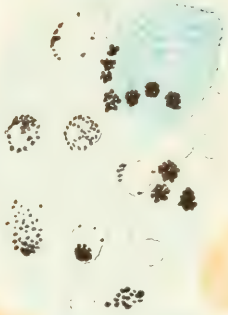


FIG. 2.



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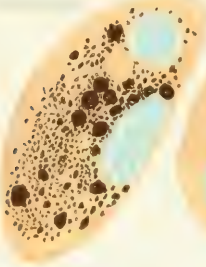

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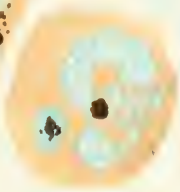

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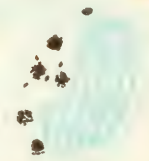

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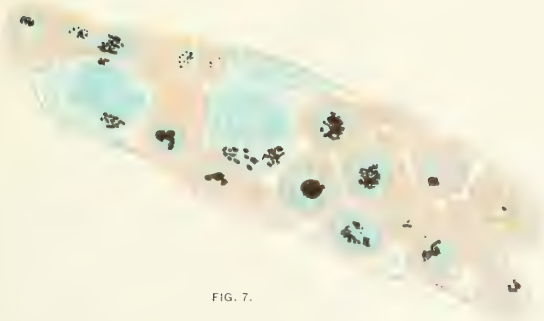

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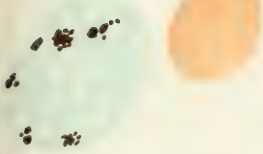

FIG. 8.

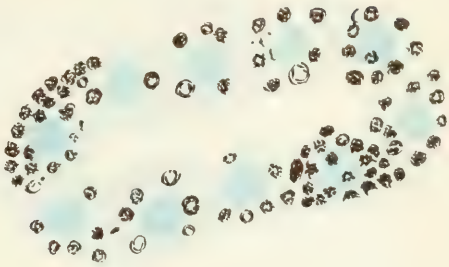

FIG. 9.

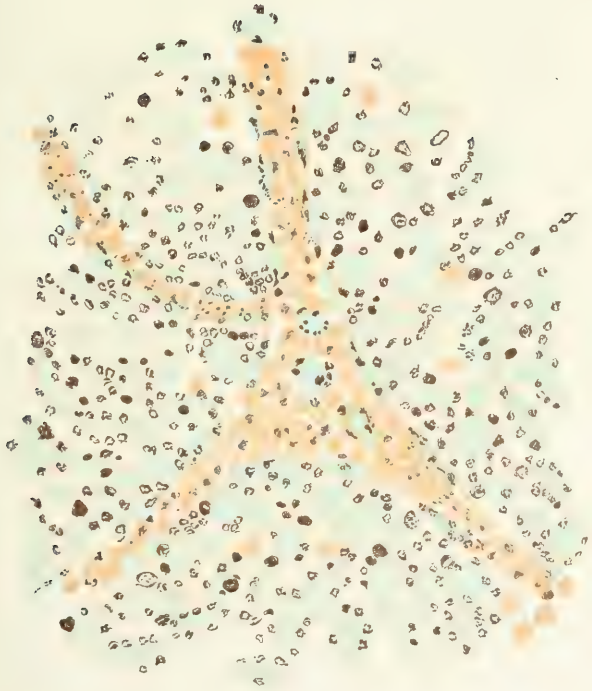

FIG. 10.

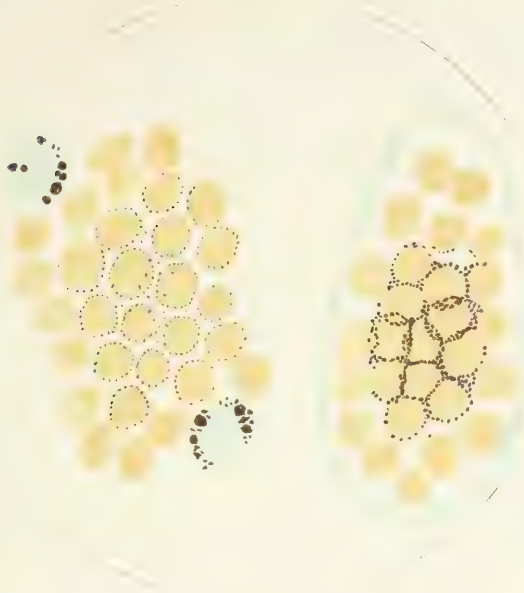

FIG. 11.

FIG. 12

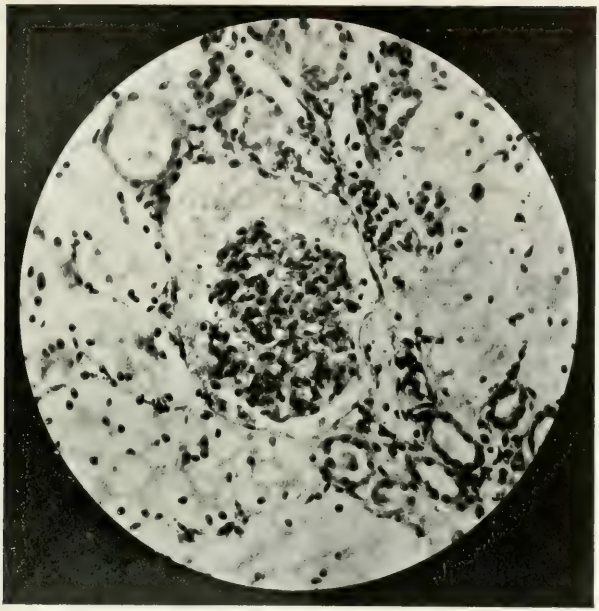

FIG. 13.

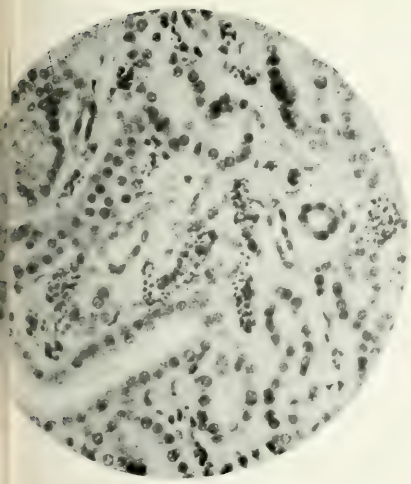

FIG. 14.

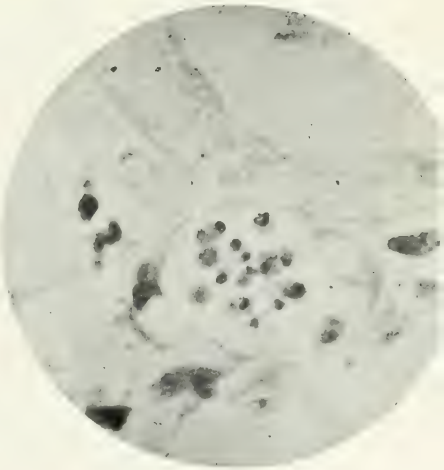

FIG. 15.

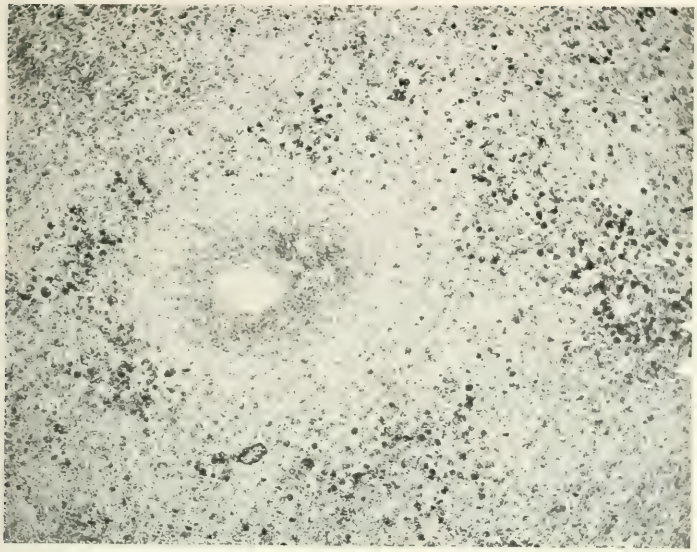

FIG. 16.

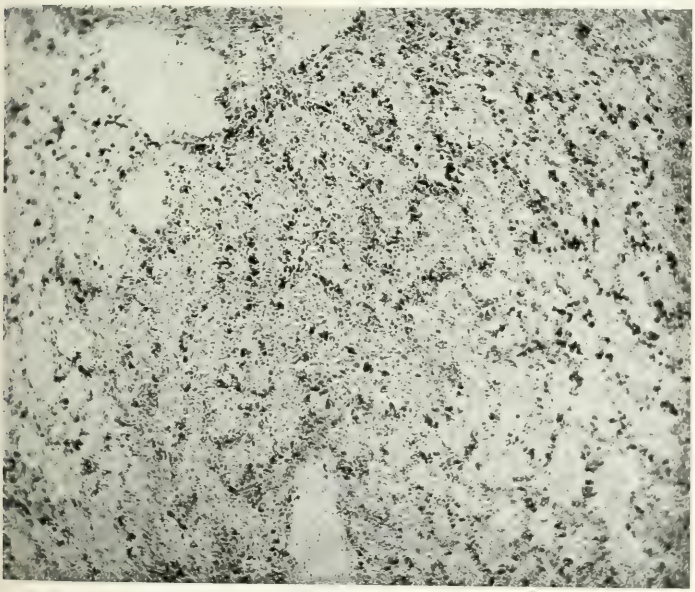

FIG. 17.

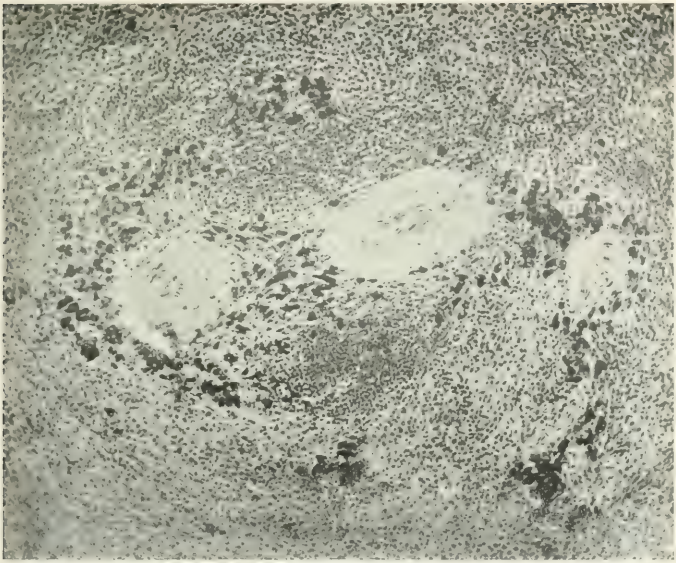

FIG. 18.

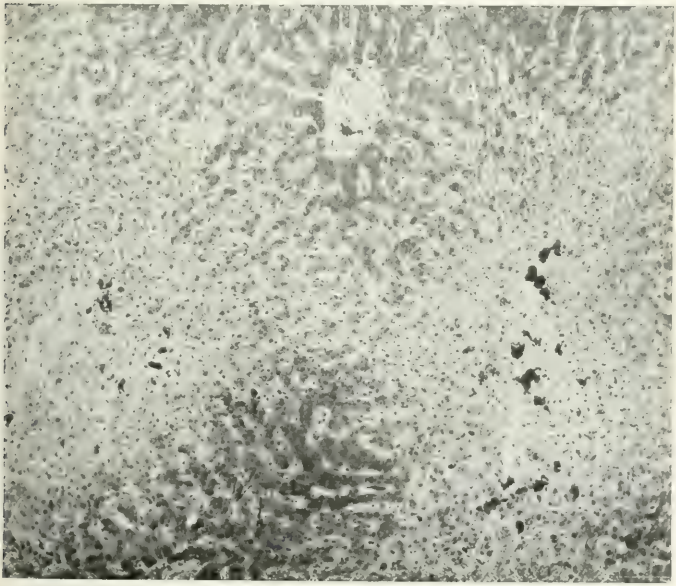

FIG. 19

[Reprinted from The Medical News, March 29, 1902.]

THE PRESENT STATUS OF SERUMTHERAPY IN TYPHOID FEVER.[1]

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In view of the very successful results that have been obtained by the serum treatment of diphtheria and the preventive therapeutics of rabies, the question naturally arises as to why no great success has followed very numerous attempts to affect favorably the course of typhoid fever by serumtherapy. On many grounds a comparison of the clinical features and pathological characters of diphtheria and typhoid fever would seem to indicate the latter disease rather than the former as the one more favorable for the successful inauguration of a system of serumtherapy.

Diphtheria is a disease which commonly develops with great rapidity and without announcing its advent by distinct premonitory symptoms. Its focal lesion in the pharynx often reaches extensive development in a few hours; its toxins are thrown into the circulation in overwhelming doses and produce grave cellular alterations in vital tissues in the first stages of the malady. Typhoid fever, on the other hand, is slowly ushered in, as a rule, by several days of premonitory symptoms or mild illness, serious anatomical lesions are not present till the second or third week, ample time is offered before either the intestine is gravely damaged or the internal viscera exhibit degenerative changes for the administration of

[1] Read before the New York County Medical Association, Nov. 18, 1901.

any agent that may have power to inhibit the growth of the bacillus typhosus or to neutralize its toxins. Yet the mortality of diphtheria has been greatly reduced by specific antitoxin treatment, while typhoid fever has long remained untouched and almost unapproached by advances in the knowledge of artificial immunization.

It may be of interest to point out some of the reasons which may be advanced to explain this failure to solve the problem of successful serotherapy of typhoid fever. In the first place it develops more clearly each year that there are fundamental differences in the nature of these two bacterial diseases which were long overlooked. In diphtheria the bacteria multiply rapidly on superficial mucous surfaces and seldom reach the general circulation, producing their effects by diffusible toxins absorbed from the focus of infection. Successful isolation of the bacillus diphtheriæ from the circulating blood is of rare occurrence, and the few cases in which it has been accomplished serve only to demonstrate the almost invariable rule that in fatal diphtheria the blood is free from the specific bacterium of the disease.

In typhoid fever, while earlier and several recent studies by Pasquale, Thiemich, Kraus, Ettlinger, Stern, James and Tuttle, Neuhaus, Fränkel, and Klein, indicated that the blood seldom yields positive culture of bacillus typhosus, very recent reports from several sources prove that with more careful technic the bacillus can be isolated from the blood in the majority of cases, even comparatively early in the disease. This conclusion has for some time been favored by the increasing proportion of successful attempts to obtain the bacillus from the cutaneous lesions. Thus, Soudakoff and Thiemich obtained the bacillus in fifty per cent. of their cases from the rose

spots, and more recently Neuhaus obtained it from the skin in thirteen of fourteen cases.

Regarding the occurrence of the bacillus in the blood Kraus and Kuhnau showed that there is a type of typhoid septicemia in which the microorganism is practically always to be isolated from the blood. During the past year several studies have shown that even in routine cases a much higher proportion of successful results may be obtained than had been regarded as possible. Thus Castellani was successful in twelve of fourteen cases. Schotmuller has contributed an important study of sixty-eight cases in eighty-five per cent. of which he found the bacillus in the blood. He distributed twenty c.c. of blood from the basilic vein in several flasks containing from 200 to 300 c.c. of broth, the dilution nullifying the bactericidal action of the blood, while the large bulk of blood tended to insure a successful result if any bacteria were present. This result emphasizes the absolute necessity of drawing conclusions in this field only from the use of considerable quantities of blood. Almost equally successful were Auerbach and Unger, who planted only from ten to thirty drops of blood in 300 c.c. flasks of bouillon. Examining the broth in hanging drops they sometimes found at first a very scanty growth of non-motile rods or threads which, when replanted on fresh broth free from blood, gave an abundant growth. With these small quantities of blood they had seven positive results in ten cases.

These studies and others of similar import which are known to be in progress prove that typhoid fever in the great majority of cases, whether fatal or favorable, is a form of septicemia; and, as many features of the disease have led us to suspect that the symptoms of typhoid fever are not referable so much to the intestinal lesions

as to the presence of the bacteria in the blood and viscera, the increasing frequency of reports of typhoid infection without intestinal lesions thus becomes quite intelligible.

Typhoid fever therefore is largely a bacteremia, while diphtheria is a toxemia.

Another important addition to the knowledge of typhoid fever has resulted from recent bacteriological studies of the blood and the investigation of the agglutinative properties of the serum, which tend to show that there is a wide variation in the characters of the bacteria concerned in the disease. Babes was among the first to point out various marked differences between cultures of bacillus typhosus isolated from typhoid cadavers. Some of them produced gas and indol, properties which tended to throw them into the colon group. He usually obtained from the same cadavers cultures of typical typhosus, and, while denying emphatically that the irregular types were capable of producing typhoid fever, he admitted that they probably influenced the character of the disease, since they acted in animals just as did typhosus. Many observers have reported extreme differences of virulence in cultures obtained from typhoid cadavers. Remy finds that when typhosus is grown along with colon bacillus the former loses its susceptibility to agglutination and may be so far altered as to be unidentifiable by any or all of our present means.

On the other hand, studies of the colon bacillus have shown that this species very frequently develops characters which render its separation from typhosus extremely difficult. Sternberg isolated several species of coli from water which failed to produce indol or gas or to coagulate milk, but were agglutinated by high dilutions (1-10,000) of typhoid serum. Deeleman also obtained colon cultures which produced no gas and

did not coagulate milk. Remy finds that when grown with typhosus the colon bacillus regularly loses its capacity to produce gas and indol. Jatta found that he was able to isolate from diarrheal stools at different periods of the disease different types of coli which varied in their capacity to coagulate milk and were agglutinated in varying degrees by blood serum.

The hope that the agglutination test would prove a ready means of separating typhosus from the colon bacillus has not been realized. Stern found that typhoid serum may agglutinate coli even more strongly than typhosus, especially colon cultures from the stools of the typhoid patients whose serum was employed in the test. Widal and Nobecourt found a patient whose serum agglutinated typhosus only in 1-20 dilution, but clumped a culture of coli from an abscess in a dilution of 1-12,000. Eight days later the serum had no effect on typhosus while retaining its power over the colon bacillus. Beco concluded that typhoid serum agglutinates most colon cultures, sometimes very actively but always less strongly than typhosus, if an extremely powerful serum be used.

This conclusion, while generally holding, has found exception in the experience of Widal, Sternberg, Remy and Jatta. Moreover, Jatta showed that this high agglutinating power of typhoid serum over the colon bacillus could not be referred to secondary infection by the bacillus coli, since it was present in the serum of animals receiving injection of typhosus only. Not only has the agglutination test failed to distinguish typhosus from coli, but it appears to be unable to separate all cultures of these germs from other more distantly related members of the intestinal flora, since it has been shown by Gruber, Durham, Sternberg, Deelman, Remy, and many others,

that typhoid serum even in comparatively high dilutions may agglutinate Gaertner's bacillus enteritidis, Nocard's bacillus psittacosis, and Petruschky's bacillus fecale alcaligenes.

The accumulating evidence of studies in these related fields seems therefore to justify Durham's division of this entire group of intestinal bacteria into three, instead of two, classes, and to warrant the common application of the term "Gaertner's group" to those species intermediate between typhosus and coli, with which the production of gas and indol and the coagulation of milk, while often present in traces, are too uncertain to warrant their acceptance either as genuine typhosus or as genuine coli. An important practical bearing of these conclusions was not apparent until bacteriological studies of the blood of typhoid fever began to show that the typical symptoms of this disease may be associated with the presence in the blood, as the undoubted etiological agent, of bacteria which produce gas and indol, coagulate milk, and are not agglutinated by typhoid serum.

The credit of having first drawn definite attention to the fact that some irregular forms of typhoid fever are referable to infections by bacteria intermediate in type between Eberth's bacillus and the colon bacillus belongs originally to several French observers who combined the results of study of the bacteria isolated from the stools and the agglutinating properties of the serum of these patients. Achard and Bensaude appear to have published the first complete description of a case of this type in 1896.

In 1897 Widal isolated from an abscess in a phthisical patient a bacillus resembling coli, but producing no indol and not fermenting lactose, while the patient's serum, which had no effect on typhosus or on cultures of genuine coli, agglu-

tinated this bacillus in dilution of 1-1,000. In 1898, Gwyn isolated from the circulating blood of a case of typhoid fever a bacillus which produced no indol and did not ferment lactose, but fermented glucose, while the patient's serum, inert with typhosus, agglutinated this bacillus in 1-200 dilution. Shortly before this Brill had described an epidemic of typhoid fever of a type which probably belonged in this class, since in none of the eighteen patients was Widal's reaction obtained. Unfortunately there were no bacteriological analyses of the blood, while cultures of the stools yielded the colon bacillus. More recently Schotmuller, in five of sixty-eight routine cases of typhoid fever, found bacteria in the circulating blood which on biological characters must be classed as intermediate between bacillus typhosus and bacillus coli. These cases were usually of mild type and not such as might be expected to show bacteremia. The blood sera failed to agglutinate cultures of genuine typhosus, but acted strongly on the bacillus obtained from the blood in each case, and all of five sera agglutinated all the cultures in the series. In about the same proportion of cases, five out of sixty-two, studied by Kurth, the diagnosis of paratyphoid inflection was made, either by the presence of specific agglutinating properties in the serum, or from the isolation from the stools, or urine, of bacteria which produced no indol, fermented glucose, and in other characters resembled Gaertner's group. These cultures lost their virulence when heated to 60° C. and were harmless when administered by mouth to guinea-pigs and rats. The serum of these patients had no effect upon bacillus typhosus nor on bacillus enteritidis, but actively agglutinated the particular cultures isolated from the stools.

Rodet recently isolated from three typhoid cad-

avers bacilli which produced no indol and no gas, but grew on potato like the colon bacillus. The initial cultures were indifferent to both typhoid and colon serum, but subcultures were agglutinated by both sera in high dilutions, most actively by the colon serum. Animal inoculations gave a serum which actively agglutinated typhosus. These apparently contradictory results belong to the class on which is based Roux's theory that the colon and typhoid bacilli are identical species with varying and interchangeable physiological properties. The exact characters exhibited by one representative of the paracolon group of bacteria, isolated from a case of post-typhoidal osteomyelitis, have been very minutely studied by Cushing.

The above observations indicate clearly the importance of recognizing the paracolon or Gaertner group of intestinal bacteria as rather frequent excitants of diseases which may be clinically indistinguishable from typhoid fever. They serve to explain the failures of Widal's reaction which are constantly being reported, and they point out the necessity for testing suspected typhoid serum both on genuine typhosus and on cultures of this paracolon group as well.

We have thus far shown that typhoid fever differs from diphtheria, first, in being a septicemia rather that a toxemia; and, second, in that the bacterial agent in the disease, not to mention secondary infections, may show wide variations in its biological characters and in the quality of the agglutinating substance which it causes to be elaborated in the system. Moreover, the toxic products of bacillus typhosus differ radically in their clinical qualities from diphtheria toxin, while their relation to the symptomatology of the disease is very uncertain. Typhotoxin differs from diphtheria toxalbumin in being slowly soluble in

water, hence resembling globulin rather than serum albumin. It differs from globulin, moreover, in being very slowly soluble in NaCl. According to Martin it does not possess the digestive properties of diphtheria toxin. Typhotoxin kills rabbits and guinea-pigs very slowly; diphtheria toxin kills them very rapidly (Breiger, Fränkel). In neither animal does it give symptoms of lesions similar to those of typhoid fever, even when acting energetically; yet Remlinger produced rather typical lesions of typhoid fever in rats and rabbits by feeding them on cabbage-leaves soaked in cultures of bacillus typhosus. Peyer's patches were swollen, there were ulcers in the colon and the spleen and lymph-nodes were hyperplastic. Recently, Walker induced typical lesions of typhoid fever in rabbits by combining succesive inoculations of typhoid cultures with immune horse serum and serum extract. Sanarelli grew typhosus in broth for a month in the thermostat, then for eight months at room temperature, finally killing and macerating the bodies of the bacilli at 60° C. for some days; 1.5 c.c. of this extract per 100 grams of guinea-pig killed in twenty-four hours, with bloody diarrhea and lesions which showed a very slight tendency of the toxin to pick out lymphatic structures, but with no specific lesions in Peyer's patches. Martin found the filtrate of virulent cultures to kill animals with subnormal temperatures, but with no specific lesions. Bodies of the bacilli killed by chloroform were much more toxic, but even then did not specially affect Peyer's patches. Bacillus enteritidis gave somewhat similar results.

We may conclude, therefore, that the bodies of typhoid bacilli contain poisons which do not diffuse rapidly in fluids as does diphtheria toxin and which are incapable of producing the specific lesions of typhoid fever. Not only has no specific

typhotoxin been isolated from test-tube cultures of bacillus typhosus or from the cadavers of typhoid patients; but Chantemesse and Widal, Gruber and Durham, and many others, have shown that the procedures which render animals immune to infection by the typhoid bacillus are incapable of protecting them against infections of the products and macerated bodies of these bacteria.

The problem of the serumtherapy of the disease is therefore the preparation of a serum which is principally bactericidal and not antitoxic as in diphtheria. It was at first believed that the agglutinating substance represented the essential principle in preventive sera in typhoid fever, but it has gradually become clear that, while the agglutinins are usually abundant in strongly protective sera, such active sera may exhibit very little agglutinating power. Pfeiffer has shown that cholera serum may lose almost all its agglutinating properties while retaining a high degree of protective power. Deutsch, among others, has made very careful observations on the development of agglutinins and of protective substances in the blood of inoculated animals, finding that the two curves are generally but not always parallel, and never superposable. He concluded that all strongly agglutinating sera are also strongly protective, but that highly protective sera may contain little agglutinin.

Although immune serum usually contains both bodies at the same time and loses both in about equal proportions when heated or inoculated with typhoid germs (Gruber and Trumpp), by a comparison of the agglutinating powers of many sera, Pfeiffer and Kolle, Fränkel and Otto, and Deutsch have concluded that these properties have no necessary relation to each other, and certainly do not reside in identical substances. Their

conclusion accords with the fact that the highly protective diphtheria and tetanus sera are not agglutinative, while the agglutinating typhoid and cholera sera cannot be made to show highly protective qualities.

Again, several studies have shown that agglutination depends to a large extent upon the characters and condition of the bacteria themselves and is only partly determined by the quality of serum with which they are treated. Johnson found that actively growing cultures of typhosus often tend to agglutinate spontaneously, while slowly growing attenuated cultures do not. Durham found that of sixteen different vibrios the less virulent races were much more susceptible to agglutinins produced by virulent cultures than were those virulent races themselves. Walker's observations seem to show conclusively that typhoid cultures showing greater tendency to spontaneous agglutination and agglutinating more strongly in immune serum are the less virulent cultures. The most that can be claimed is that the agglutination is not the essential feature of protective serum, that agglutination depends very often upon special tendencies of the bacterial species rather than upon the presence of much bactericidal substance in the serum, but that agglutinative power is, however, in the majority of cases, a fairly reliable index of the protective power of a serum.

Serumtherapy in typhoid fever therefore demands the preparation of a principle which is not largely antitoxic, which indeed is not to be measured by its agglutinative powers, but one which responds to the far more critical test of protection by a mechanism which has thus far largely escaped recognition. This means probably that it must be both antitoxic and bactericidal. The evidence that both these properties are demanded

may be gathered from the results, on the one hand, of Brieger, Kitasato, Wassermann, Beumer, Peiper, Sirotinin, Bitter, and Bruschetini, all of whom prepared protective sera which were more or less antitoxic, and, on the other hand, of Pfeiffer and Kolle, Funk and Bockenham, and Sanarelli, whose actively protective sera were slightly or not at all antitoxic.

We may now consider briefly what steps have been taken with or without regard to the foregoing principles and what success has been achieved in the preparation of a protective serum for typhoid fever in the human subject. Bockenham inoculated horses, first with the filtrate of typhoid cultures, then with the killed cultures, and then with both alternately, thus securing a serum of considerable bactericidal power and with active protective and curative powers in animals. Pope and Cowen tested this serum on the human subject in a few cases with results which seemed to them more favorable than those secured by any other plan of treatment of the disease. Immediate improvement followed each injection and the course of the fever appeared to be distinctly shortended. Bockenham's serum was prepared, however, without special regard to some theoretical requirements. The first of the theoretical difficulties was recognized by the endeavor to produce a polyvalent serum, *i.e.,* one that would protect against a considerable variety of races, each with special characters, which, as has been shown, may be concerned in the disease. Each of these races seems to require an extremely specialized bactericidal agent and some may be little affected by a serum which rapidly destroys other races of bacillus typhosus. This difficulty was foreshadowed by the conflicting results of Widal's test, the serum of some convalescent patients failing entirely to agglutinate particular cultures of typho-

sus, at least partly because the serum did not contain the special agglutinin for that culture, although probably quite capable of agglutinating a bacillus which came from the patient's own intestinal tract.

This idea of the necessity of a polyvalent serum was first suggested by Bockenham in connection with the manufacture of antistreptococcus sera. The idea was followed up systematically by Tavel and Walker, who inoculated horses for a period of from one to three years with several species of bacillus typhosus, finding that the specialism in the immunizing bodies required to protect is very marked and that greater specialism is demanded as the protective qualities increase. These results probably indicate the real difficulty in the prepation of practically effective sera for the human subject. Although Walker secured enormous agglutinative powers in his horses (1-2,500,000), this power was exhibited only against the particular culture longest employed in the inoculations. Against other cultures its agglutinative qualities were far less and proportional to the length of treatment by each culture. Tavel tested serum drawn after one year's treatment of horses, on typhoid patients, finding that it abolished the plateau stage of pyrexia, the fever falling by lysis and the patients making rapid recovery. Other cases treated by two-year serum are soon to be reported, and three-year polyvalent serum is now being tested (Walker).

A second practical step in advance followed the recognition that the protective substances are not equally distributed in the body. Johnson and McTaggert found that the whole blood possesses more agglutinins than the serum. Wassermann (1899) tested the protective qualities of extracts of the different organs and concluded that the typhoid antibodies are produced in

the spleen; but almost at the same time Roth's studies in a similar line led to negative conclusions. Jatta's studies indicated that within from two to three days after inoculation the agglutinins of the spleen exceed those of the blood serum, but later the serum is always the stronger. He did not test the protective qualities of the spleen and his results were amplified by Castellani, who found that while the serum contains more agglutinins the spleen has more protective bodies.

Deutsch took up the matter in a systematic and exhaustive manner. He found that the spleen and marrow usually show greater bactericidal power than the blood serum, which he attributed to the abundance of leucocytes in these organs. Yet in splenectomized animals bactericidal properties developed as in the normal animals, indicating that the function of the spleen may be fully assumed by other tissues. Bactericidal substances made their appearance on the fourth or fifth day of infection, simultaneously with the disappearance of leucocytes from the peritoneum, the site of inoculation. Removal of the spleen on the third day greatly reduced the animals' chances of recovery apparently through the removal of already formed bactericidal substances. The lymphatic organs contained less agglutinin than the serum. Agglutinins developed perfectly in splenectomized animals, but splenectomy on the third to fifth day of infection sometimes prevented their formation. He found marked discordance between the bactericidal and agglutinating properties of the spleen, and hence concluded that these properties reside in different substances. Deutsch made the interesting discovery that lung tissue in the early stages of infection has from ten to twenty times as powerful agglutinating action as the serum. Later, the difference was not so great. Normal lung was also

found to be from ten to twenty times as active as normal serum of guinea-pigs.

These researches of Wassermann and Deutsch represent the acme of progress in the investigation of immunity in typhoid fever, and they culminate, as have many previous studies, with strong indications that immunizing bodies are the product chiefly of the colorless blood and lymph-cells. Daubler's partly contrary results in this field, however, here deserve mention, since this investigator found that during the process of immunization against typhoid infection the leucocytes and purulent exudates do not increase in bactericidal properties, but remain the same throughout.

The above results have to some extent received a practical test on the human subject in the attempt of Jez to influence typhoid fever by injections of visceral extracts from infected animals. Proceeding from the principle that the normal central nervous tissue possesses both preventive and curative properties in tetanus, Jez tried extracts in glycerin, alcohol, salt, broth, and water, of the liver, spleen, lymph-nodes, thymus, marrow, brain and cord, of healthy rabbits, administering them subcutaneously and by mouth, but without effect on typhoid infection of rabbits. He then inoculated rabbits with 10 c.c. of typhoid culture, the animals dying on the fourth day. Extracts in alcohol and water of the thymus, spleen, marrow, brain, and cord proved actively preventive, when 10 c.c. were injected with 10 c.c. of culture. An extract of the above tissue made with alcohol, water, salt, and glycerin, to which a trace of carbolic acid and pepsin was added (details not given), had no bactericidal action in the test-tube and gave no Widal reaction; but when given hypodermically is said to have had a surprising effect upon the course of typhoid fever in eigh-

teen cases on which it was tried. The fever was reduced, pulse improved, sweats induced, and the general nervous condition much improved. Glucksmann failed, however, to obtain any curative action from Jez's extract on typhoid infection in rabbits, and Walker, replacing Jez's extractive solution with curative horse serum, failed to find any greater curative powers than were obtained from horse-serum alone. Jez's results lack, perhaps, the scientific certification of the work of Tavel and Walker, but represent a line of work in the right direction.

Another independent line of investigation has been caried on by Chantemesse in Paris, from which especially successful results have been expected, inasmuch as this investigator claimed, in 1898, to have devised a method capable of furnishing extremely active and specific typhotoxin. Chantemesse cultivated the bacillus upon the filtrate of an emulsion of splenic tissue digested with pepsin, carefully avoiding access of air during the manipulations. The fluid thus secured was found to be extremely toxic, especially for horses, but on exposure to air rapidly lost its poisonous properties. Horses were very susceptible to minute doses for at least one year, after which they developed increasing immunity and their blood serum became strongly bactericidal and both curative and preventive for animals. The results of the treatment of 100 cases of typhoid fever with Chantemesse's serum have recently been reported. The total mortality was 6 per cent., which is not better than the results obtained in many such series of cases treated by ordinary methods. Yet two patients began treatment on the twenty-first and twenty-fifth days when already suffering from pneumonia with a pulse-rate of 140. A third case was that of a very fat woman who could not be bathed; she died

of a large bedsore present on admission. Three of the patients died of perforation, against which the treatment seemed of little avail. On the other hand, all patients treated before the tenth day recovered. The serum usually caused a rapid and steady defervescence. Or the temperature rose again, the relapse being more severe than the original fever, but a second injection caused a rapid lysis of fever. The injection of the serum invariably caused a pronounced improvement in the general symptoms, as attested by all the clinicians who observed the course of Chantemesse's treatment in the Paris hospitals.

It remains for investigators who possess experience and extensive equipment to combine and extend the results already obtained in the two aforementioned fields and to determine what success can be secured in the treatment of typhoid fever by injections of serum and visceral extracts from animals which have been treated with a view to producing polyvalent immunizing bodies. It would appear that the outlook in this field is fairly indicative of success, if not in the preparation of a curative serum for typhoid fever, then at least in essential progress in the knowledge of artificial immunization both in this disease and in other infections which have heretofore baffled all attempts at serumtherapy.

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PRELIMINARY REPORT ON A STUDY OF SMALLPOX.

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During the past year I have studied from autopsies at North Brother Island, New York City, twenty-nine cases of smallpox. Access to this material was obtained through the kindness of Dr. H. M. Biggs, Dr. W. H. Park, and Commissioner Cosby, of the New York Department of Health, and to these gentlemen I desire to express my great obligation, as well as to Drs. Ray and Berry, resident physicians at the Island.

The general plan followed in undertaking the study of a disease with which I was entirely unfamiliar was the following:

1. The pathologic anatomy.
2. The histology.
3. The routine bacteriologic study of the heart's blood and cutaneous lesions by ordinary methods.
4. The same field treated by special methods of bacteriologic study.
5. Experimental variola.

In the first three of these departments the work has been nearly completed, while in the last two only a beginning has been made.

I. Pathologic anatomy.

The Cutaneous Lesions. One may divide these lesions into the following classes:

1. Primary hemorrhagic smallpox, fatal on the second and third days, before the development of general discrete variolous lesions, and marked by diffuse hemorrhages into the skin and mucous membranes. This is the most severe type of the disease, and its violence is most appalling. The series include three such cases.

2. Secondary hemorrhagic smallpox. (Eight cases.)

Here the usual variolous lesion is early complicated by hemorrhages into the skin and mucous membranes, and sometimes these hemorrhages are so early and diffuse as to constitute a transitional variety between primary and secondary hemorrhagic smallpox.

In addition to the usual type of this variety, in which the hemor-

rhages occur early in the disease, one hemorrhagic case was encountered in a "bleeder," in which the early pustules became filled with blood, and a general punctate hemorrhagic eruption appeared on the sixth day. Also on the ninth day of another case the usual well-filled pustules suddenly became the seat of a distinctly secondary hemorrhage, producing a true "black smallpox."

3. Confluent or conglomerate smallpox. (Nine cases.)

This is the most common type of the disease, and is often fatal.

4. Discrete smallpox. (Seven cases.)

This type of the disease is usually benign, but a good many cases die from complications.

5. In a newborn infant dying at fourteen days the eruption consisted of about a dozen large blebs scattered over the trunk.

Two interesting variations of the usual cutaneous lesions were observed. While various stages of the eruption are often to be found in the same cadavers, in one case were exhibited two distinct crops, one consisting of large hemorrhagic pustules, the other appearing several days later as very numerous small vesicles. In another case a scanty discrete scabbing lesion was complicated in convalescence by a diffuse scarlatina-form eruption. Possibly this was scarlatina grafted on variola, but no further cases developed in this children's ward, and in another such case there was in due time a typical scarlatinal desquamation.

Respiratory Mucous Membranes. I examined the upper nares in a few cases, but was disappointed in the extent of the process found, which was distinctly less than in the pharynx, although variolous ulcers and diphtheritic membrane were encountered. The posterior nares, fauces, and pharynx usually showed a profuse eruption ulcerating early and frequently becoming diffuse and diphtheritic.

The trachea usually presented the most severe lesion in the cadaver. In eighteen, or practically all of the acute cases, there was a diffuse diphtheritic lesion extending from the epiglottis to the finest bronchioles. In the primary hemorrhagic cases there was diffuse hemorrhage and very superficial diffuse necrosis. If not fatal, this lesion, or a milder one, is succeeded or replaced by a catarrhal or purulent bronchitis, as found in five cases dying late in the disease. In three cases with mild discrete cutaneous lesions the trachea showed discrete pustules. In one remarkable case the trachea exhibited very numerous early lesions of pure conglomerate variola. Twice the respiratory mucosa showed a severe and active process when the lesion in the skin was scanty and nearly healed. One diphtheritic case failed

to survive tracheotomy on account of extensive edema of the trachea and neck compressing the air passages.

The conclusion was reached that the lesion of the respiratory mucous membrane is, as a rule, earlier than that of the skin, and far more important in causing death.

Lungs. Seven cases failed to show any consolidation in the lungs. They died late in the disease, without much involvement of the trachea. Two cases showed a late lobar pneumonia. All the others exhibited some grade of lobular pneumonia, which was usually the chief immediate cause of death. The intensely black, congested, edematous lungs of the hemorrhagic type of the disease are practically pathognomonic. A fibrinous pleurisy was occasionally seen.

Heart. Three cases showed acute ulcerative endocarditis. One developed this lesion from the first, and died on the eighth day. One developed the lesion as the skin was healing, and one was convalescing and out of bed before the cardiac symptoms were detected.

Liver. Various grades, usually marked, of acute degeneration were regularly noted.

Kidneys. Acute degeneration was the usual lesion. Once acute exudative and productive nephritis was found. In three primary hemorrhagic cases there were some minute hemorrhages throughout the organ. In the pelvic epithelium I found no specific variolous lesions, but failed to look for them with unusual care. In the primary hemorrhagic cases the pelvis showed diffuse hemorrhagic and diphtheritic inflammation. The urine in these cases was very bloody.

Aside from petechiæ I found no lesions in the bladder.

The Gastrointestinal Tract. Several cases showed numerous variolous ulcers of the epithelium of the esophagus. In the primary hemorrhagic cases there was more or less diffuse diphtheritic process in the esophagus. In five cases there were numerous minute variolous ulcers in the stomach, and once there were large areas of diphtheritic necrosis. Petechiæ were common. In one confluent hemorrhagic case there was marked hemorrhage from the intestines and a diphtheritic enterocolitis, affecting the entire colon and lower ileum. In the primary hemorrhagic cases the stomach and entire colon, but not the small intestine, showed diffuse infiltration of the mucosa with disintegrating blood.

Lymphatic System. Extreme congestion and swelling of the nodes was a constant gross sign. The swelling of the mesenteric and gastrointestinal lymphatic structures was very marked and in proportion to the intensity of the disease. The spleen was large, firm in the early

stages, drained of lymphocytes and flabby in the late stages, sometimes exhibited hemorrhages and focal necroses, and often a peculiar mottled condition which I have not encountered in other diseases.

The marrow showed the same variations in its cellular contents, and was more often the seat of hemorrhages.

Testis. I found three cases of acute necrotic inflammation of the testis, and it may have been more common, as the testes were not always examined even in the gross. In one confluent case the urethral epithelium was untouched. In seven females I found no specific lesions in the uterus or vagina.

Eye. There were conjunctival and bulbar hemorrhages in the primary hemorrhagic cases. No variolous lesions were to be seen in the conjunctival epithelium. In some cases they might have escaped observation.

The middle ear looked normal in two cases.

Brain. The brain and meninges were congested and edematous when examined, but were not touched in the primary hemorrhagic cases.

II. Histology. The histologic examination of the tissues in these cases has led to the conclusion, stated by Weigert many years ago, that the first effect of the variolous process in ordinary intensity is coagulation necrosis, followed by liquefaction and exudative phenomena. In the epithelial tissues the lesion shows a marked focal character, often becoming diffuse. In some other tissues, especially in the spleen, marrow, and testicle, the lesions are apt to be focal. In the lungs the focal lesions develop by extension down the air passages. In other tissues the lesions are those of a diffusible toxin. The hydropic changes so prominent in the cutaneous vesicle seem to be secondary to coagulation necrosis, and to result from exudation into the necrotic area. This conclusion is not in accord with my preconceived notion that the virus of smallpox must be one which primarily liquefies tissues.

Another prominent histologic character is the extreme congestion attending the local process. I have never seen anything else approaching the remarkable distention of all the vessels of the respiratory submucosa of an ordinary case of smallpox. This same congestion about cutaneous lesions causes the frequent hemorrhagic character of the eruption. In the primary hemorrhagic cases all the cutaneous and mucous surfaces are literally drenched with blood.

A third main histologic character is the exclusive preference of the lesion for epithelial tissues, which seems to indicate that the virus requires epithelial albumin for its propagation.

Only the bladder and small intestine, possibly also the uterus and vagina, seem to escape involvement. On the other hand, the parenchyma of organs, as the spleen and the testicle, may show a somewhat specific localized process.

The Blood. The blood was examined in twelve cases, with results similar to those reported in the recent elaborate study of Courmont and Montagard (*Journ. de Phys. et de Path. gén.*, 1900, pp. 557, 781). The presence of from 30 to 50 per cent. of large and medium sized mononuclear cells and some myelocytes, with distinct leukocytosis and active fever, is a rather pathognomonic sign of the disease. It is worth while pointing out that the high proportion of mononuclear cells is frequent at a period when the cutaneous exudate is highly purulent and when the pustules commonly yield cultures of streptococcus pyogenes.

In the primary hemorrhagic cases there is during life beginning dissolution of the red cells, with hemoglobinemia, and after death the dissolution continues, so that in sections of tissues there may be abundant deposits of pigment similar to those seen in puerperal septicemia of streptococcus origin.

I had hoped that the examination of both the blood and tissues by Nocht's method, which seems especially adapted to the demonstration of protozoa, might yield some interesting information. The results obtained by a laborious study of all the cases by this method were entirely negative, as I found no traces of protozoa.

III. Bacteriologic. The plan of the study included the bacteriologic examination of the heart's blood and cutaneous lesions: first, by the ordinary methods, and, later, by any special methods which the previous study might suggest.

a. Systematic examination of smears of the cutaneous lesions in all stages, stained by various methods, led to the following conclusions:

1. Vaccine bodies are present in all stages of the specific lesions, and their numbers are proportional to the hemorrhagic character of the lesion.

2. Streptococci are present in all the specific lesions at a very early stage, frequently in enormous numbers, but very often scantily, and sometimes cannot be detected in the smears of vesicles and even of pustules.

3. The next most common bacterial form encountered in smears is a fine bacillus resembling in general a minute Klebs-Loeffler. It corresponds to the bacillus described by Klein and Nakanishi in vaccine. It is discussed later.

4. Smears of variolous lesions always contain a great variety of minute bodies resembling bacteria, cocci, or bacilli, which cannot be positively identified as bacteria, but which open up wide possibilities for the demonstration of a bacterial origin of the disease.

b. Cultures upon blood serum and other media were made in all cases from the heart's blood, and usually from several vesicles in the skin.

A streptococcus was obtained from the heart's blood in all of the twenty-nine cases. Twice it was associated with Klebs-Loeffler in cases showing diffuse diphtheria of the pharynx and larynx, and in two cases, both dying late in the disease, with much ulceration of the skin, staphylococcus aureus also grew.

The abundance of the growth of streptococcus in the heart's blood was directly proportional to the severity of the disease, and was also influenced to a less extent by the time intervening between death and the postmortem.

It was least abundant in cultures taken after death in cases dying in the early stages of the eruption, and in such cases, to ensure a successful culture, at least ½ to 1 c.c. of blood should be planted.

After death it multiplies rapidly in the body. In hemorrhagic cases it is more abundant, and in three primary hemorrhagic cases a score of chains of streptococci were found in each oil-immersion field of the thinly spread heart's blood four to six hours after death, while the cultures were extremely profuse. It was also very abundant in the blood smears of a newborn infant dying on the fourteenth day, with very scanty bullous eruption.

I made five examinations of 10 c.c. of blood taken from the basilic vein during life. They were all sterile. Arnaud (*Rev. de Méd.*, 1900, p. 303), however, found streptococci in the circulating blood in two primary hemorrhagic cases. Only one of my five cases died, and it was not of the primary hemorrhagic type.

Cultures of the skin were made in twenty-three cases. The skin was first pencilled with 5 per cent. formalin for two minutes, then in sterile water, and the cover of the vesicle removed with sterile instruments. A platinum needle was then touched to the base of the lesion and the material transferred to blood serum, etc.

In seventeen cases streptococci grew alone, once with staphylococcus aureus, once with a fine bacillus (Bac. 19), once staphylococcus aureus only was obtained, and three cases gave no growth. In one of the sterile cases streptococci were afterward found in sections of the skin. In several cases other bacteria grew in isolated colonies, which were set aside as contaminations.

Seeing that the streptococcus was found by this crude technic in nearly 90 per cent. of the cases, I feel justified in concluding that it is invariably present in the cutaneous lesions as well as in the heart's blood. If the skin be allowed to remain at room temperature for twenty-four hours I believe the streptococcus can always be isolated from it by culture, which, of course, would not prove it present there during life.

The streptococcus showed many peculiarities, all of which may characterize this micro-organism as obtained from other sources. It grew best on blood serum, feebly or luxuriantly; it sometimes clouded the broth in initial, but never in subcultures; its chains were very short, except in ascitic broth, in which the chains were very long. The individuals varied from an extremely minute size to 3 to 4 μ in diameter. It sometimes grew in bacillary form, as described by Hektoen, from diphtheria, and sometimes it split lengthwise instead of sidewise. Under the skin of mice it once killed in four hours. When rubbed into the shaved ear of a rabbit it killed in forty-eight hours with general septicemia. Sometimes it was less virulent for rabbits.

Inoculations. Material from smallpox pustules was inoculated into rabbits, into the conjunctiva, skin, and veins; into guinea-pigs by the same routes; into rats subcutaneously, and into two monkeys by vaccination. The monkeys—one a *rhoesus* and the other a large African black-faced fellow—both yielded a rather typical form of inoculated variola with discrete pustules, the scab dropping after two weeks. All other experiments were negative.

The streptococcus was inoculated repeatedly into rabbits, pigs, rats, one calf, and two monkeys. It sometimes killed the small animals, but never produced anything like variolous lesions in any of them.

Bacillus 19 was a minute bacillus obtained from the skin of Case 19, and once later from the heart's blood. It sometimes exhibited cross striations like a minute Klebs-Loeffler. After remaining inoculated in a calf two weeks it appeared in smears of pus as a minute coccus, but regained its bacillary form on first cultivation. It grew as an almost transparent film on agar and blood serum, and its chief character was the active fluidification of blood serum and gelatin. It reminded one so strongly of the bacillus isolated by Martin from vaccine, and claimed by him to be the specific contagium of cowpox, that I gave it considerable attention.

It killed a rabbit in forty-eight hours with septicemia when rubbed into a scarified area of the skin. A calf kindly placed at my dis-

posal by Dr. Park was vaccinated, with a negative result. It was then injected beneath the skin of this calf, producing an abscess containing the germ as a coccus, pure; and the first attempt to produce vaccinia in this calf failed, but a second extensive vaccination gave a "dry take." On three monkeys it had no effect whatever. Smallpox blood serum agglutinated the bacillus but feebly. This bacillus was finally inoculated, together with streptococcus, in a calf and a monkey, always without specific lesion.

From vaccine and from the skin of a case of variola I obtained Nakanishi's bac. variabilis, which gave negative results when vaccinated on calves, monkeys, and small animals.

Lastly, I have inoculated the streptococcus with the macerated skin of smallpox sterilized by heating to 75° C. for one hour on eight consecutive days. This treatment will destroy many but not all ferments, and will leave unaffected many albumoses. The results were negative.

Conclusions. At the present time, while it hardly seems possible that the streptococcus is the specific contagium of variola, one cannot fail to be strongly impressed by the fact that this micro-organism is present at all stages of the disease and in numbers proportional to its severity.

It is difficult to reconcile the principles governing secondary infection by streptococcus with the presence of this bacterium in the blood during life, and with its enormous numbers in the blood very shortly after death of the primary hemorrhagic cases. In these cases it cannot be doubted that the streptococcus completely overshadows any other bacterial agent that may be present, and the idea of a secondary infection in such cases does not seem reasonable. I believe that the condition in these cases should be regarded rather as a symbiosis of the streptococcus and the specific agent, from a very early period, so early as to be indistinguishable in these cases from a primary invasion.

While not inclined to urge that the streptococcus is the specific agent in variola, I believe it will always remain impossible to reproduce the disease, especially in its fulminant types, without the aid of this micro-organism.

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PARATYPHOID INFECTIONS:[1]

WITH REPORT OF A CASE CLINICALLY IDENTICAL WITH TYPHOID FEVER IN WHOSE BLOOD A PARATYPHOID BACILLUS WAS FOUND.

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Since Gärtner's discovery of the B. enteritidis, in 1888, increasing interest has attended the study of the intermediate members of the typhoid-colon group of bacilli. In 1893 Gilbert proposed the terms "paratyphoid" and "paracolon" to designate these intermediates; but Durham regards the terms as inappropriate, since he believes that the intermediates are well-defined species of organisms, and suggests that all be classified under the group name "Gärtner and its allies." From the clinical stand-point, however, the term paratyphoid is the most clearly descriptive that can be applied to infections resembling typhoid and caused by one of these intermediate bacilli.

According to Durham, the Gärtner group includes B. enteritidis and the similar organisms described in association with epidemics of meat-poisoning, the gas-producing "typhoid" bacilli which have been described by various observers, the B. psittacosis, the paracolon bacillus described by Gwyn, and the bacillus O (Cushing). Theobald Smith has shown that B. choleræ suis and B. typhi murium should be classified with this group, and Cushing suggests that the recent researches of Reed and Carroll on the B. icteroides of Sanarelli demonstrated that it, too, belongs here. In addition, Widal adds the bacillus of calf septicæmia of Thomassen.

Pathogenicity in the human subject has been established for certain members of the Gärtner group—*e. g.*, B. enteritidis in epidemics of meat-poisoning, B. psittacosis in a peculiar epidemic bronchopneumonia with typhoid symptoms in some cases, and the paratyphoid bacilli in cases resembling typhoid fever. Moreover, Grunbaum has suggested that febrile jaundice may be caused by one of the intermediates, since he has obtained the Widal reaction with sera from these cases.

Petruschky's bacillus faecalis alkaligenus, according to Durham's latest work, is not an intermediate of the typhoid-colon group, but occupies

[1] Read at a meeting of the New York Pathological Society, February 12, 1902.

a place beyond the typhoid in that it does not acidify any sugar-containing media; in a sense it out-typhoids the typhoid bacillus.

In the present paper we shall concern ourselves chiefly with cases clinically resembling or identical with typhoid fever which were apparently caused by an intermediate bacillus.

In 1896 Achard and Bensaude reported two cases which they considered due to infection with paratyphoid bacilli.

Case I.—Woman, aged twenty-four years. Had never had typhoid fever. For several weeks had nursed a child ill with a "mucous fever." Taken sick August 24th with a chill, followed by fever, anorexia, slight diarrhœa, marked prostation, and tympanites. No spleen, no epistaxis, no rose-spots. Temperature, 103.3° F. No râles in the chest. Intestinal hemorrhage. September 11th, marked pyuria. September 14th, temperature fell suddenly to 98.6° F. without apparent cause, remounting to 100.4° F. September 20th, temperature 98.6° F., rising to 102.2° F. on the 23d, with a return of the diarrhœa and the development of right femoral thrombosis. October 2, left femoral thrombosis. October 5th, temperature normal.

Blood from vein at elbow remained sterile. Urine taken aseptically September 12th and 23d, both times gave a bacillus which was considered to be a paratyphoid. Blood aspirated from the spleen showed staphylococci, but no bacilli. Stools gave colon only.

September 9th, no Widal. September 14th, the serum reaction with same typhoid culture and the bacillus obtained from the patient's urine was positive. September 17th, two cultures of typhoid bacilli gave the reaction. October 16th, positive Widal with three of thirteen cultures of typhoid bacilli. Reaction constantly positive with paratyphoid. Constantly negative with colon. Seven weeks after the fever the patient's serum agglutinated the bacillus obtained from the urine and two cultures of paratyphoid bacilli, but was without action on five cultures of typhoid. The blood of animals immunized with the bacillus from the urine reacted with paratyphoid bacilli, and only with certain samples of typhoid bacilli.

The authors think the case presented a close analogy to typhoid fever complicated by pyelonephritis, with a relapse complicated by femoral thrombosis.

Case II.—Infant, aged seven months. June 23d, temperature was 100.4° F., with subcrepitant râles over middle of left lung posteriorly. Temperature reached 104° F. on the 26th. The pulmonary signs disappeared, but the fever continued between 102.2° F. and 104° F. Tympanites, rose-spots in abundance. July 13th, temperature 98.6° F. Discovered swelling of right sterno-clavicular articulation, which was opened and a paratyphoid bacillus recovered from the pus. Patient recovered completely.

The authors conclude that while general infection with B. typhosus cannot be excluded in this case they can affirm positively that the local infection was paratyphoid in nature.

In discussing the paper, Rendu states that the authors have demonstrated that paratyphoid fever closely resembles typhoid in its course,

temperature curve, and abdominal symptoms. Cases of psittacosis infection, he says, may present the general appearance of typhoid fever, but show special pulmonary localization. He has seen three cases. All three presented signs of grave bronchopneumonia. None had diarrhœa, rose-spots, or tympanites. One died. There were no intestinal lesions at the autopsy.

In the following year, in his thesis, Bensaude contended that the bacilli isolated from these cases were B. psittacosis not paratyphoid as originally reported, but his bacteriological evidence only proves that the bacilli were intermediates. He did not conclusively differentiate the bacillus of psittacosis from paratyphoid bacilli. Moreover, he seems in doubt as to the real nature of the general infection in the two cases.

In 1897 Widal reported the following case: A tubercular patient, after three weeks in the ward, developed an abscess in the neck about the œsophagus, presenting at the same time slight constitutional symptoms. A bacillus was obtained from the pus, which, from its cultural characteristics, Widal considered a paracolon. The bacillus gave a marked reaction with the serum of the patient in dilutions as high as 1 : 1000, the reaction steadily diminishing with convalescence. Widal concludes that the bacillus isolated was the cause of the patient's infection. The sera of animals inoculated with B. coli communis and with B. psittacosis gave no reaction with this organism.

Gwyn isolated, in 1898, from the blood of a patient suffering with a disease clinically identical with typhoid fever a bacillus which culturally corresponds with Widal's. The patient had been ill since September 17th, with headache, fever, weakness, and later with vomiting, diarrhœa, and pain in the abdomen. October 16th he had three hemorrhages from the bowel. He slowly rallied and went out of the hospital in five weeks. Rose-spots were present, the spleen was palpable, the urine gave the diazo-reaction. The temperature was that of a severe typhoid. The Widal reaction was never found. Blood cultures taken October 12th gave a paracolon bacillus. The patient's serum reacted with this bacillus in dilutions of 1 : 150 to 1 : 200. Two months after the date of the culture there still remained a slight reaction. The same serum was without action on B. typhosus in any dilution above 1 : 1 to 1 : 5.

Cushing's case followed in August, 1900. M. B., colored, aged twenty-seven years. From early in June, 1898, nine months before admission, the patient had suffered with a prolonged course of fever extending over a period of ten weeks. Clinically, it was typhoid fever, with a severe but typical course. There was a relapse ushered in by profuse epistaxis. During convalescence two tender swellings developed near the breast-bone. One disappeared, the other burst, dis-

charging a large amount of pus and leaving a sinus at the level of the fifth rib. Microscopical examination showed the process to be an osteomyelitis. There was no Widal reaction. Cultures taken from the sinus at the time of the operation gave an intermediate bacillus, which Cushing calls bacillus O.

In the same month appeared Schottmueller's first case. Male, aged twenty-six years. The patient was taken ill July 23d with headache and catarrh of the upper respiratory passages. Temperature 104° F. July 27th, the patient was sleepy and tired; spleen palpable. July 28th, the sixth day of the disease, the blood furnished a bacillus resembling the typhoid, but fermenting glucose. Rose-spots present. Temperature normal, August 5th. The only symptom of typhoid lacking was the stools. August 1st the Widal reaction was negative, while the bacillus isolated from the blood reacted with the patient's serum at 1 : 50, but not in 1 : 100. Later the reaction was obtained at 1 : 100. With three other typhoid cultures the reaction was negative. Of the sera of four typhoid patients only one gave a reaction with the bacillus, and that at 1 : 20.

In 1901, Schottmueller reported five more cases, clinically like typhoid fever, in whose blood he found paratyphoid bacilli. None of the cases gave the Widal reaction. There was a sixth case which did not give the Widal reaction from whose blood he failed to obtain a bacillus, but this may be explained by the fact that the blood was drawn only three days before the end of the fever.[1]

Case I.—Male, aged sixty years. Taken ill July 7th with headache, lassitude, diarrhœa. Spleen not palpable. No spots. Temperature, 104° F. Temperature normal August 1st. Mild type.

Case II.—Male, aged eighteen years. Taken ill July 13th with headache, etc. Temperature 105.2° F. July 18th, condition bad. July 28th, condition dangerous. September 18th, discharged cured. Severe typhoid type.

Case III.—Male, aged nineteen years. Taken ill August 16th with headache, etc. Temperature, 104.3° F. Rose-spots present. October 1st discharged cured. Very mild type.

Case IV.—Male, aged forty-six years. Taken ill about August 12th with headache, etc. Had been in bed six days before admission to hospital. Rose-spots present. Spleen palpable. Temperature 103° F. Discharged cured December 14th. Mild type.

Case V.—Male, aged fifteen years. Admitted November 3d. For fourteen days had been ill with fever, lassitude, etc. Temperature 105.4° F. Many rose-spots, which appeared on the face and hands as well as in the usual situations. Patient in bad condition until November 12th. December 30th he was discharged cured. Severe typhoid.

Case VI.—Male, aged twenty-five years. The patient was a physi-

[1] In a limited number of cases of typhoid fever we have failed to find the bacillus in the blood after the second week, and it will be noted in our case of paratyphoid infection that the blood drawn on the twenty-seventh day of the disease remained sterile.

cian who had assisted in the examination of Case II. Temperature 101.6° F. Dismissed cured September 16th. The blood did not give the Widal reaction. Blood taken three days before the end of the disease remained sterile. The diagnosis of typhoid fever was made by exclusion.

There were no complications with any of the cases, even the severe ones. Case IV. was alcoholic, Case I. was sixty years of age. In these cases, the author says, one would expect a bad prognosis in true typhoid fever, yet both were mild.

The six cases above recorded occurred in a series of sixty-eight cases which Schottmueller examined, a high proportion, if we can judge by the comparative frequency of the Widal reaction in typhoid fever.

Schottmueller applies the term paratyphoid both to the infection and to the bacillus.

Kurth also published, in 1901, five cases which he considered paratyphoid fever. These cases were found among sixty-two whose sera were tested for the Widal reaction.

Case I.—Woman, aged thirty years. Taken sick May 17th. Temperature 103.2° F. Normal June 11th. Tendency to constipation. Widal negative except in dilution of 1 : 3. June 30th the bacillus was recovered from the urine.

Case II.—Male, aged twenty-nine years. Taken sick August 27th, having had headache for a week, with fever and diarrhœa. Splenic tumor, rose-spots, diazo-reaction. Temperature 100.4° F. to 104.9° F. No complications. Widal negative three times. Bacillus isolated from stools September 19th. The patient's serum reacted with this bacillus at 1 : 8000, and on October 28th at 1 : 2000.

Case III.—Male, aged twenty-five years. Taken sick September 3d with headache and vomiting. Splenic tumor, few rose-spots, diazo-reaction. Temperature up to 104° F. Widal reaction was tested three times, and was positive once at 1 : 3. No paratyphoid bacilli found either in stool or in urine. The patient's serum reacted with the bacillus from Case II. at 1 : 500.

Case IV.—Male, aged eighteen years. Taken sick October 26th. Typhoid stools. Temperature 98.6° F. to 104° F. Normal November 27th. Rose-spots, splenic tumor. Widal negative twice. The patient's serum reacted with the bacilli from Cases I. and II. at 1 : 500.

Case V.—Woman, aged twenty-three years. Entered hospital November 7th. No splenic tumor; doubtful rose-spots; no diazo-reaction. Irregular temperature, never above 102.2° F. Serum reaction positive.

All the cases were mild. None had complications. In Cases III. and IV. the cultures were not taken till late in the disease, which may explain the negative result.

In the course of a study on the bacteriology of the blood in typhoid fever we encountered the following case :

Louise R., colored, aged twenty-eight years, entered Bellevue Hospital in the service of Dr. Loomis, October 9, 1901. She gave the

following history: For the last two months she had been living in Elizabeth City, N. C., and was taken ill one week after her return to New York. On October 4th the patient had a chill which lasted about half an hour and was followed by fever and sweating. Her appetite has been poor and she has had frequent attacks of vomiting, the vomitus consisting of the food last eaten. There have been two attacks of epistaxis. Since October 6th she has suffered from severe headache. Her bowels have moved freely, with a slight tendency to diarrhœa. The patient feels very weak. Temperature on admission was 103.2° F., pulse 92.

Physical examination on admission. The patient is well nourished, the muscles are firm, the skin is moist and elastic. The mucous membranes are red. The tongue is tremulous, fissured, coated over the dorsum, red at the tip and edges. The pulse is small, frequent, regular, and of low tension. The heart is negative. The lungs are negative except for slight impairment of resonance posteriorly. The abdomen is soft, but gives a tympanitic note. Liver normal. Spleen not palpable. Slight epigastric tenderness. Owing to the patient's color we could not determine the presence of rose-spots.

The patient had two stools on each of the first two days in the hospital, but had been given a dose of castor oil. Subsequently there was slight constipation.

An examination of the urine, made October 10th, showed a trace of albumin, with granular and hyaline casts, blood, pus, and epithelial cells. A leucocytic count October 11th gave 10,600. The same day the blood was examined for the malarial parasite, with a negative result. The Widal reaction, tested by Dr. T. W. Hastings, was tried three times: October 10th, dilution 1 : 80, negative after one hour; October 16th, dilution 1 : 40, negative after half an hour; October 31st, dilution 1 : 80, negative after one hour. With another culture of typhoid bacilli an imperfect and only partial reaction was obtained at the end of an hour in a dilution of 1 : 20. On October 9th, the sixth day of the disease, and on October 30th, the twenty-seventh day of the disease, 10 c.c. each of blood were taken from a vein at the elbow for bacteriological examination. The first of these specimens gave a paratyphoid bacillus, the second remained sterile.

Clinically the patient followed the course of a mild attack of typhoid fever. The spleen became palpable as the disease progressed. The subsequent history was uneventful except for a sharp break in the temperature to 99.6° F. on the fourteenth day of the disease, after which the exacerbations and remissions were very marked till the twenty-second day, when the temperature fell to 98.6° F. and convalescence was definitely established. The patient was discharged cured November 20th, six weeks after entrance to the hospital and seven weeks after the beginning of her illness.

Rose-spots were sought for many times, but because of the patient's color it was impossible to decide if they were present.

To sum up: The illness was ushered in with a chill, followed by fever, anorexia, lassitude, slight diarrhœa and fibrillary tremor of the tongue. The temperature curve was continuously high to the fourteenth day of the disease, when it fell to 99.6° F., after which the curve was broken by exacerbations and remissions to the twenty-second day,

the first day of convalescence. Added to these symptoms there was splenic tumor. So that in all essential features the case was typical of mild typhoid fever.

Analysis of the Symptoms of Paratyphoid Fever. In view of the care with which the cases from whose blood the paratyphoid bacilli have been isolated were studied, it must be admitted that these organisms are capable of causing a general infection that has to be reckoned with in the diagnosis of typhoid fever. An analysis of the symptoms of the reported cases demonstrates that this infection closely resembles true typhoid infection, and may even be identical with it in clinical manifestations. Practically all of the cases of paratyphoid infection passed clinically for typhoid fever, without a Widal reaction, until careful bacteriological examination revealed their true nature.

In so far as conclusions can be drawn from the limited number of cases reported, paratyphoid infections may be mild or severe as in genuine typhoid. The symptoms of invasion are practically the same, for we may find in both headache, lassitude, anorexia, diarrhœa, epistaxis, chill followed by fever, and slight inflammation of the upper respiratory passages.

Moreover, during the course of paratyphoid infection the patient develops the typhoid state, slight or profound, accompanied by tympanites, diarrhœa or constipation, rose-spots, splenic tumor, and quite a typical temperature curve. The pulse is that of typhoid fever. The urine sometimes gives the diazo-reaction.

Abscess of the neck, osteomyelitis, pyelonephritis (?), and intestinal hemorrhages have been reported as complications. Even a relapse occurred in Cushing's case. So that the picture is more or less complete as a whole.

For the most part the cases have been mild, and Schottmueller states on the evidence of his seven cases that the prognosis is favorable.

Certain irregular typhoid cases have been reported in which the Widal reaction was absent, which we are inclined to regard as examples of paratyphoid infection. Brill has made a careful study of seventeen of these cases. In our case there was a remarkable break in the temperature curve, without assignable cause, to 99.6° F. on the fourteenth day of the disease, though the temperature afterward remounted to 101.8° F. Possibly some of the cases of the so-called fourteen-day type of typhoid fever are really paratyphoid fever, but we have been unable to obtain any data on this point. Dr. Thomas D. Coleman, of Augusta, Georgia, writes us that the fourteen-day typhoid fever is quite common in that section, and that in all essential symptoms it is identical with the twenty-one-day type. He also states that the Widal reaction was negative in one of these cases, but a bacteriological examination of the blood or feces was not made.

Brill's cases bore a marked clinical resemblance to typhoid fever, though the duration of the disease was only ten to twelve days. The serum reaction of these cases was tested with typhoid bacilli daily, with negative results. The symptoms, briefly stated, were: anorexia, malaise, headache, and general body pains for three or four days, succeeded by rigors or a chill, and sometimes epistaxis. The temperature rose rapidly to 104° F. to 105° F., after which a mild typhoid state developed. Rose-spots were generally present. The spleen was enlarged. Constipation was the rule. The temperature reached normal ten or twelve days after the beginning of the illness. Inconstant diazo-reaction. No leucocytosis. No intestinal hemorrhage. No complications or sequelæ.

In three of the cases cultures were made from the feces and B. coli communis obtained. In one case the spleen was aspirated, and again B. coli communis found. The serum of each case was tested for a reaction with the colon bacillus and found negative. And in two cases the serum of the patients was tested with colon bacilli obtained from themselves, with no reaction. In these two cases then the bacilli could not have been paratyphoid bacilli.

Case XIV. of Brill's series is especially remarkable. The patient had had typhoid fever, with the Widal reaction, six months previously and had entirely recovered. The serum reaction against the typhoid bacillus persisted up to and including part of the second illness, during the course of which it disappeared. Brill justly claims that the second attack was not typhoid fever.

The cases reported as fatal typhoid fever with intestinal lesions, not showing the Widal reaction, without bacteriological examination are not proved to have been genuine typhoid infection.

The Widal Reaction in the Light of Infections by Members of the Gärtner Group of Bacilli. Since the introduction of the serum reaction as a means of diagnosis in typhoid fever it has been a well-recognized fact that a small proportion of cases which are clinically typhoid fever fail to give the reaction. Brill, adding to Cabot's statistics, finds that of 4879 cases 4781, or 97.9 per cent., gave the reaction. Gwyn gives 99.6 as the percentage of positive reactions in the Johns Hopkins Hospital. On the contrary, in all the reported cases of paratyphoid infection the reaction has been absent. It is probable, then, that some, at least of the typhoid cases with negative reaction, were really paratyphoid. That not all of them were paratyphoid is made probable by the following remarkable case reported by H. Batty Shaw:

Adult. Headache for three days. Temperature 103° F. Typhoid fever was suspected, and on the two following days the Widal reaction was tried at 1 : 50, and both times failed. On the seventh day of the disease rose-spots appeared and remained visible until the nineteenth

day. Splenic tumor. Delirium and incontinence for three weeks. Temperature normal the twenty-fourth day. An abscess developed in the region of the right rectus abdominis. It was incised the thirty-sixth day of the disease and a typhoid bacillus isolated from the pus. The patient's own serum in dilution of 1 : 200 failed entirely to clump this bacillus after an hour's exposure. Another typhoid case in the hospital, giving the Widal reaction with another typhoid culture, failed to react with this bacillus at the same dilution. The serum of the patient was then tried with a typhoid culture from University College, but in dilution of 1 : 100 gave only the slightest clumping after twenty-four hours. The same culture with the serum of another patient with typhoid fever gave the reaction in two minutes.

The bacillus isolated corresponded to the following biological and other tests: 1. Decolorized by Gram. 2. Actively motile in broth—no scum. 3. Shake preparation in glucose-agar, no gas. 4. No indol in broth after ninety-six hours. 5. Growth on potato present, but not visible. 6. Did not curdle milk.

No adequate explanation offers itself in this case, except some biological peculiarity which was not demonstrated. It scarcely seems possible that the bacillus isolated was not the cause of the patient's infection.

It cannot be assumed that all cases, clinically typhoid, which have been reported as giving the Widal reaction were cases of genuine typhoid infection. The brilliant work of Durham on the typhoid colon group and their serum reactions has established the fact that certain members of this group may be mutually interacted upon by sera of infected patients and immunized animals. This is especially true of sera in low dilution, and since in the earlier years of the Widal reaction the technique had not been worked out, and dilutions were more frequently low than not, some of the cases reported as typhoid fever may have been paratyphoid. The simple fact that clumping of bacilli has been obtained is not sufficient evidence for diagnosis. Gruber and Durham have proved that a serum reaction is not in the strict sense *specific*, that it is only a *special* reaction, and that it indicates not a certainty, but a probability.

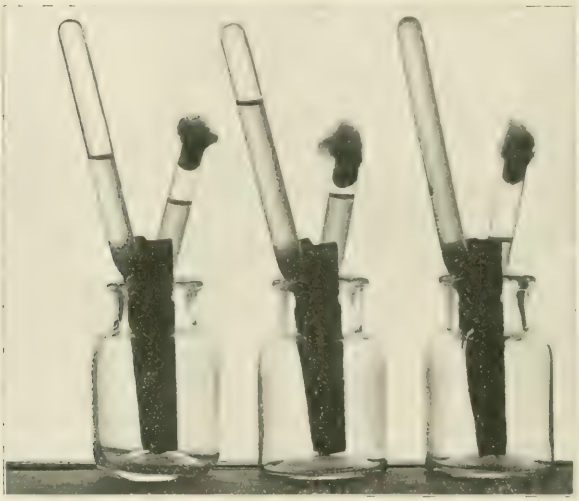
Landsteiner was the first to observe that typhoid serum reacts positively against Gärtner's bacillus enteritidis. Durham has confirmed this observation. In his study of an epidemic of meat-poisoning at Oldham he found that the sera of 5 of 29 cases of infection with B. enteritidis gave minimal traces of reaction against the typhoid bacillus at 1 : 100, and in lower dilution some of them gave well-marked clumping with typhoid cultures. Hence, he states that "in the absence of differential tests they might have been returned as having been obtained from typhoid fever patients." In another place he makes an even stronger statement based upon tables he gives, that, for the cases examined, in dilutions "lower than and including 1 : 100 it is imma-

terial whether true typhoid or Gärtner's bacillus is used to obtain the typhoid reaction." He adds that "sera obtained from typhoid fever patients are not potent enough to give absolute indications either for diagnosis of bacilli or the nature of serum."

In our case the serum reacted with the typhoid bacillus at 1 : 20, though only partially and imperfectly at the end of an hour, and failed entirely at 1 : 40 and 1 : 80 with another typhoid culture. In Gwyn's case his paracolon was agglutinated by typhoid sera of high potency

FIG 1.

Gas formation in 2 per cent. glucose broth.



Coli communis. Case 7. Typhoid.

(1 : 300 to 1 : 1100) in dilutions up to 1 : 5. One typhoid serum (potency 1 : 900) gave an incomplete reaction at 1 : 30.

Moreover, Gilbert and Fournier and Achard and Bensaude have shown that B. psittacosis is agglutinated by typhoid sera at 1 : 10, but not at 1 : 40 to 60.

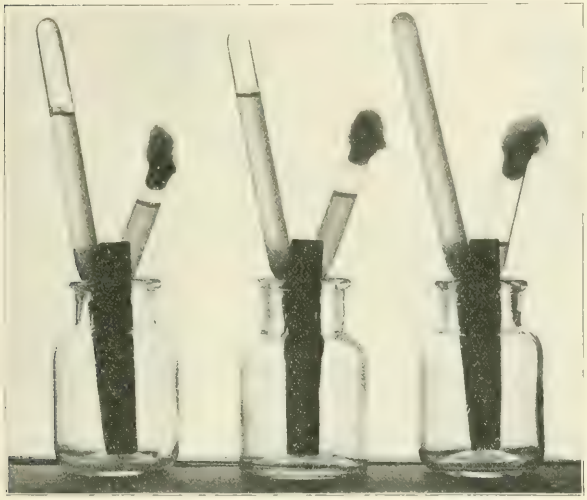
Hence, it cannot be too strongly insisted upon that the ordinary routine test for the Widal reaction give place to more reliable and scientific methods. Dilutions of sera should be high, rarely or never less than 1 : 50, and in the absence of a positive reaction the serum

should be tested against several of the intermediates. Not until this is done will the data of the serum reaction in typhoid fever possess scientific value.

Bacteriological Report. Although the bacteriological work on the bacillus in question, here called Case 7, has not been completed, enough has been done to show that it unquestionably belongs to the group intermediate between coli communis and typhoid, and that among the intermediates themselves it resembles Cushing's bacillus O

Fig. 2.

Gas formation in 2 per cent. maltose broth.



Coli communis. Case 7 Typhoid.

and Gwyn's (paracolon) bacillus—Durham's group C—more nearly than the B. enteritidis of Gärtner, hog cholera and others—Durham's group D.

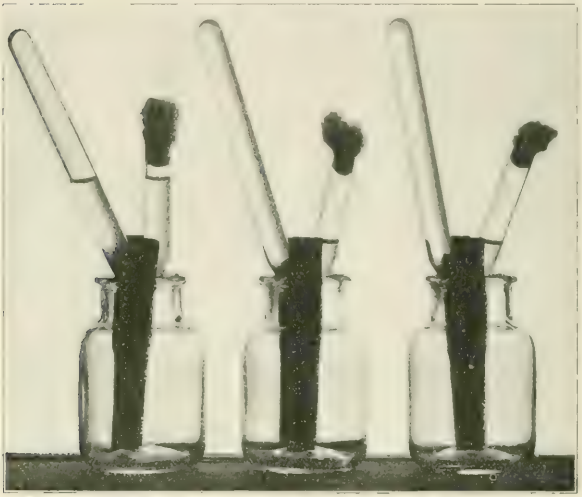
Of the 10 c.c. of blood drawn from the median basilic vein, 4 c.c. was distributed into four Erlenmeyer flasks, each containing 50 c.c. of broth; the remaining 6 c.c. being dropped into six tubes, each one containing 6 c.c. of broth. The flasks and tubes were incubated, and the following day two of the flasks appeared turbid. On the third day the other two flasks and one of the tubes were cloudy, but the remaining

tubes showed no growth either then or later. This experiment and many other similar ones undertaken by us have shown the necessity for considerable dilution of the blood if positive results are to be looked for. Cole has discussed this point very fully.

The turbidity of the four flasks and one tube was found to be due in each instance to a pure culture of the same bacillus, which at first was taken to be typhoid in spite of the fact that the patient's blood gave no Widal reaction with typhoid cultures, even in low dilutions. This

FIG. 3.

Gas formation in 2 per cent. lactose broth



Coli communis. Case 7. Typhoid.

absence of reaction at that time was ascribed to the early stage of the disease, fifth or sixth day, in which the blood was taken. On testing the bacillus, however, in glucose gelatin it was found to produce gas, and this led to further investigations.

In ordinary media, agar, gelatin, and broth, the growth is indistinguishable from typhoid, nor do stained preparations, ordinary or by Gram's method, show any differences.

Hanging Drop. The bacillus is very motile, even more so than typhoid usually is, and in this resembles the bacilli isolated by Schott-

mueller, Kurth, and others. Kurth remarks that his bacilli possess flagella which are larger than those of typhoid and do not show the same peritrichal arrangement. In our bacillus, however, the flagella appear to be precisely similar to those of typhoid.

Serum Reaction. The bacillus was hardly affected by serum of the patient in the early (fifth, or sixth day) stage of the fever, but serum taken on the twenty-seventh day, at which time cultures from the blood were negative, agglutinated the bacillus in high dilutions, reacting toward typhoid cultures only in low, one to twenty dilutions, and even then, slowly and more or less imperfectly.

Later on the dried blood of the patient agglutinated cultures of Cushing's O and Gwyn's paracolon, but in somewhat lower dilutions. The blood of a rabbit immunized to Case 7 by repeated subcutaneous injections reacted strongly with the bacillus in high dilutions, and also with O and Gwyn, but was entirely negative with cultures of typhoid.

It seems, however, doubtful if the serum reaction will prove of value in distinguishing the intermediates as an entire group from typhoid on the one hand and coli communis on the other. It is true that Schottmueller found that his bacilli (six) would interact for the most part and that Kurth reports the same with his two cultures, but Durham prepared a highly potent Gwyn serum which reacted 1 : 20,000 with Gwyn, but had no effect upon O in 1 : 100 dilutions, and Cushing himself observed that the serum of his O patient reacting 1 : 8000 with O bacillus had but slight effect upon Gwyn's bacillus at 1 : 10.

These observations so far as O and Gwyn are concerned are at variance with our own, but we find with Durham that interactions between the members of the enteritidis group cannot be depended upon: Cultures which could not be distinguished either morphologically or biographically when tested by serum for agglutination showed very marked differences.

Gas Formation. The photographs show, better than a description could do, the gas formation in glucose and maltose and the absence of it in lactose broth.

In this respect Case 7 is typical of the intermediates, all observers agreeing that they do not form gas in lactose or saccharose media. So far as saccharose goes the gas test is of no value, since many true colon bacilli are also unable to ferment it. Durham, indeed, divides the colon bacilli into two groups, coli communis verus, which does not, and coli communis communior, which does ferment saccharose. The photographs show less gas formation on the part of Case 7 than of coli communis. This difference, however, is not constant, and the same may be said of the other intermediates so far tested.

Milk is not coagulated. Schottmueller lays great stress on the fact that among the intermediates there is initial acidity in the milk, fol-

lowed by alkalinity, which in two or three weeks may reach such a point that the casein is dissolved and the milk becomes opalescent. Cushing observed the same thing. This partial clearing was found to occur with B. enteritidis and O, but not with Gwyn or Case 7.

Potato. The growth is slight and there is no discoloration, so on this medium the bacillus resembles typhoid.

Litmus whey is a medium on which Durham places great reliance, but experiments with Case 7 have led to no particular results. It clouds the whey slightly and turns it acid, while typhoid leaves it clear and forms comparatively little acid, but trials so far have not shown that the intermediates form alkali later on, as claimed by Durham, and by itself it does not seem as if litmus whey would be of much value for differentiation.

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A COMPARATIVE STUDY OF THE BACILLI INTERMEDIATE BETWEEN B. COLI COMMUNIS AND B. TYPHOSUS.

B. H. Buxton.

In the fall of 1901 Dr. Warren Coleman and I undertook to make a series of cultures from the blood of typhoid patients in Bellevue Hospital.

Very early in our investigations we came across a bacillus from a supposed mild case of typhoid without Widal reaction, which proved to belong to the intermediate or paratyphoid group.

This led to the production of a joint paper read by Dr. Coleman before the New York Pathological Society on February 12, 1902, and subsequently published in the June number of the "American Journal of the Medical Sciences."

The main part of this paper was devoted to the clinical aspects of the paratyphoid infections, the bacteriological properties of the bacillus in question, here called Case 7, being also briefly touched upon.

Instigated by the possession of this bacillus, I collected a number of intermediates from various sources with the object of making a comparative study of them. My best thanks are due to those who so kindly supplied the cultures.

I am indebted for many of them to Drs. Cushing and Johnston, of the Johns Hopkins Hospital; to Dr. Schottmüller, of Hamburg, for two; to Drs. Libman and Hewlett, of New York, who each sent me a culture of the bacilli isolated by them. In addition to this a culture was received from the Hygienic Institute at Bremen, of one of the two bacilli isolated there by the late Dr. Kurth.

Into the details of the various cases it hardly seems worth while to enter. They have all been published with one or two exceptions, and references to them are given at the end of the article.

It can be gathered from the table that a large number of the cases were typhoidal in nature, the bacilli having been obtained for the most part from the blood.

Of the unpublished ones, Strong's case I understand was not typically typhoid, whilst Malcomb was a secondary infection after true typhoid, the bacilli having been isolated from the urine during convalescence.

The clinical picture of Libman's was that of cholecystitis complicating typhoid fever. The bacillus was obtained from the gall-bladder and twice from the blood during life, but since there was a positive Widal to typhoid at one to two hundred and fifty it was thought that there was probably a mixed infection. That the bacillus actually caused the typhoidal symptoms is therefore a little doubtful, but the doubt, having been here expressed, will not be referred to again, and it will be taken for granted that the bacillus was the cause of the typhoidal symptoms.

The following is a table of the organisms examined:

	From	Isolated by	Obtained from	Case
1. Coli Communis		Buxton	Feces	Normal.
2. Hog Cholera	Moore Ithaca.			
3. Typhi Murium	Johns Hopkins.			
4. Gaertner B. Enteritidis	Johns Hopkins	Gaertner, 1888	Feces	Meat poisoning.
5. Hatton	Johns Hopkins	Durham	Feces	Meat poisoning.
6. Bac. O.	Johns Hopkins	Cushing	Abscess	Typhoidal
7. Gwyn	Johns Hopkins	Gwyn	Blood	Typhoidal.
8. Strong	Johns Hopkins	Strong	Spleen	Typhoidal.
9. Malcomb	Johns Hopkins	Johnston	Urine	Typhoid
Badach	Johns Hopkins.	Johnston	Blood	Typhoidal.
11. Milewsky	Johns Hopkins	Johnston	Blood	Typhoidal.
12. Noonan	N.Y. Hosp.	Hewlett	Blood	Typhoidal.
13. N	Mt. Sinai Hosp.	Libman	Blood	Typhoidal.
14. Müller	Hamburg	Schottmüller	Blood	Typhoidal.
15. Seemann	Hamburg	Schottmüller	Blood	Typhoidal.
16. K	Bremen	Kurth	Feces	Typhoidal.
17. Case 7	Bellevue Hosp.	Coleman & Buxton	Blood	Typhoidal.
18. Typhoid, 4 strains.				
19. Dysentery, 4 strains.				

A few remarks on some of the above may here be inserted. The culture of B. coli communis was selected as a typical one after comparison with several different strains. All these appeared to be precisely similar except that some of them fermented saccharose and others did not. Durham calls the former variety Coli Communis Communior, since he considers it to be more frequently found in the feces than the latter, which he calls "Coli Communis Verus." The bacillus chosen here as a representative ferments saccharose, so belongs to the "Communior" group.

Schottmüller during an epidemic of typhoid in Hamburg in 1900 isolated intermediate bacilli from the blood of six patients. Two of these, Müller (No. 14) and another, differed somewhat both culturally and in their agglutinating properties from the other four — Seemann (No. 15) and three others — and Schottmüller suggests that he may have been dealing, not with one, but with two nearly related groups of bacilli.

Kurth, during a typhoid epidemic in Bremen in 1900, isolated intermediates, one from the feces and the other from the urine in two cases which did not respond to the Widal test. In three other cases where the blood of the patients was negative to typhoid he obtained positive results with his intermediates. Which of these two was sent to me I do not know, but from Kurth's descriptions they appear to have been identical in every respect.

Four strains of typhoid were carefully examined and found to differ only in respect of the rapidity with which they were agglutinated. A stock laboratory culture originally obtained from the New York Health Board was selected for most of the comparative tests, since it agglutinated with typhoid serums more readily than any of the other three, all of which had been recently obtained from the blood of typhoid patients by Dr. Coleman and myself.

Four strains of the B. dysenteriæ — those isolated by Shiga in Japan, Flexner in Manila, Kruse in Germany, Seward in New Haven — were also brought in for comparison, but these bacilli, although apparently identical, do not seem to

form part of the connecting links between B. coli communis and B. typhosus, and must be considered rather as side shoots of the group. According to published accounts, they resemble typhoid except that they are not motile and are not agglutinated by typhoid serums. They are not referred to in the article.

The Bacillus fecalis alkaligenes of Petruschky, from feces, appears also to bea side shoot from the group.

It is easy to distinguish the intermediates as a group from B. coli communis on the one hand and typhoid on the other.

The main points of difference are as follows:

	B. coli communis.	Intermediates.	B. typhosus.
Coagulation of milk	+	—	—
Production of indol	+	—	—
Ferm. of lactose with gas	+	—	—
Ferm. of glucose with gas	+	+	—
Agglutination by typhoid serum	—	—	+

When, however, the intermediates are more closely studied they are found to differ to a considerable extent among themselves, both culturally and in their agglutinating interactions.

As regards those which are pathogenic for man we find them clinically divided into three groups according to the symptoms produced.

(*a.*) The meat-poisoning group. (Gärtner, B. enteritidis.) Sudden onset soon after ingestion of the meat, symptoms at first those of toxin poisoning followed by invasion of the system with the bacilli. Duration of illness four or five days, after which quick recovery. In a few cases death in two or three days.

(*b.*) The pneumonic or psittacosis group.

This has been described by several French authors, Achard et Bensaude among others, and is ascribed by them to a bacillus first isolated by Nocard from a parrot supposed to have been the original cause of an epidemic. No cultures of this B. psittacosis could be obtained and it will not be

considered. Durham puts it with the Gärtner or Enteritidis group so far as its cultural characteristics are concerned.

(*c.*) Those causing typhoid-like symptoms.

We might then be inclined to divide the intermediates into: I. The paracolons which do not cause typhoidal symptoms, including the meat-poisoning and psittacosis bacilli, together with those which are not pathogenic for man such as hog cholera, B. typhi murium, etc.; and — II. The paratyphoid sub-group, to include all those bacilli which cause typhoid-like symptoms.

When, however, we come to consider the intermediates from the bacteriological standpoint we find that this division will not, without modification, hold good, for some of those which cause typhoid-like symptoms cannot be distinguished culturally from certain bacilli of Group I., whilst there are others which can be so distinguished.

The main object of this paper is to give in some detail the comparative experiments made, and to see if any satisfactory bacteriological classification can be arrived at. It must be understood that most of the tests, especially in cases where there seemed any prospects of their assisting in the classification, were made, not once or twice, but several times so as to eliminate, as far as possible, sources of error.

A few of the less important methods of differentiation may be touched upon to begin with.

Morphologically the intermediates cannot be distinguished among themselves, nor with any degree of certainty from B. coli communis or B. typhosus.

Biologically the differences on ordinary media such as agar, serum, gelatin, and bouillon are only those of degree and cannot afford much assistance for diagnosis.

Potato is notoriously an uncertain medium to deal with, and comparative trials were found to be misleading rather than helpful. As a rule those intermediates which are classified later on as Group I. grow less freely than B. coli communis, but the growth is distinctly visible, whilst Group II. resembles typhoid; but these differences cannot be relied upon.

INDOL FORMATION.

Cushing found that his Bacillus O would produce traces of indol in nine or ten days when grown in sugar free broth, but on repeating the experiment he could detect no indol. Peckham observed that sugar free broth was superior to Dunham's peptone solution for experiments on the production of indol, so after a few negative trials with the latter sugar free broth was chosen for systematic comparative tests, which, however, were not carried on far, since it was obvious from the first that they would not assist in classification. One test was made in the following way:

Six tubes were inoculated each day for ten days in succession with B. coli communis, Gärtner, O, Gwyn, Case 7, and B. typhosus, and on the eleventh day all tested at the same time. It was found that B. coli communis began to show indol at the end of forty-eight hours; the intensity increasing day by day until a deep red color was produced. None of the others gave the reaction.

Two tests were made of the whole series with different lots of broth, one tube of each organism being grown for ten days. Malcomb gave reactions fully equal to those of the control B. coli communis, but none of the others showed more than a trace of color, not sufficient to register as positive.

Although a few authors claim to have observed slight indol formation by some of the intermediates, the majority have failed to confirm this, and it is evident that the indol test is unreliable at the best.

GROWTH ON SCRAPED-OFF CULTURES.

Widal and Nobécourt and some other French authors affirm that this is a useful method of distinguishing the intermediates as a group from the B. coli communis and B. typhosus. They tested it with a paracolon obtained from an abscess, Thomassen's bacillus of calf septicemia, and B. psittacosis. On growing cultures of these on agar for three days and then scraping off the growth they found that B. typhosus would not grow on the medium, but B. coli com-

munis grew quite freely. On the other hand, their three intermediates would grow on scraped-off typhoid cultures, but not on colon cultures, nor would they grow among themselves under these conditions. Achard and Bensaude obtained less positive results, but appear to think the method of value.

Cushing made some trials on these lines, but with very indifferent success, and my experiences coincide with his. Both serum and agar cultures were tried with very conflicting results which are not worth tabulating. Absolutely no assistance was rendered by them towards classifying the intermediates among themselves, and even as applied to the entire group the method appears to be of doubtful value.

SEMISOLID MEDIA.

Trials with five per cent gelatin and Hiss' semi-solid gelatine agar were made, both in culture tubes and by plating out, but with negative results.

Hiss' most recent medium for plating out was also tested.

Agar . . .	15 grammes.
Meat extract . .	5 grammes.
Glucose . . .	10 grammes.
Water . . .	1000 cubic centimeters.

Although this medium is undoubtedly valuable for distinguishing between colon and typhoid, colonies of the intermediates usually resemble those of typhoid too closely to be distinguished from them with any certainty. No constant difference between the intermediates themselves could be observed.

Pathogenesis.

Every one who has at any time isolated an intermediate has tested its pathogenic properties and a review of all this work makes only one thing clear, and that is that it has advanced our knowledge — not one iota.

Experiments on the pathogenicity of the intermediates were therefore not attempted.

MILK.

Milk is not coagulated by any of the intermediates, but some of them, after initial acidity, produce by degrees sufficient alkali to dissolve the casein and so render the milk opalescent. This subsequent alkalinity does not occur with typhoid, the cultures becoming acid to litmus and remaining so permanently.

The alkali production occurs only in the presence of air, and Cushing, growing intermediates in fermentation tubes, found that the milk remained acid in the closed arm whilst the alkali production was confined to the bulb.

He noticed that his Bacillus O and Gwyn's paracolon bacillus produced much less alkali than the, at that time much better known, Gärtner group, and his photographs show that Gwyn's bacillus in four weeks appeared much less translucent than Bacillus O, although he does not mention this in the text.

Schottmüller lays great stress on the alkali production for the recognition of intermediates, but of his six cases, Barg and Müller only appeared slightly opalescent after several weeks, whilst the other four became decidedly so in two to four weeks. Durham has observed the same thing, but holds that the opalescence is of little value for diagnosis, since many other kinds of bacilli will cause a gradual clearing of milk.

However, granted that certain bacilli are intermediates, it is evident from Schottmüller's and Cushing's observations that there are differences among them in milk cultures, and it is worth while to consider if these differences will assist in grouping them. Several tests were made and the results found to be constant.

Coagulation in forty-eight hours, B. coli communis.

10 days. — Slightly opalescent. — Hog cholera, Typhi murium, Gärtner, Hatton, Strong, Malcomb, Libman's X, Seemann, Kurth.

20 days. — Distinctly opalescent. — Hog cholera, Typhi murium, Gärtner, Hatton, Strong, Malcomb, Libman's X, Seemann, Kurth. Average alkalinity five-tenths per cent phenolphthalein.

20 days. — Slightly opalescent. — Bacillus O. Neutral.
20 days. — Opaque. — Gwyn, Badach, Milewsky, Noonan, Müller, Case 7. Average acidity one per cent. Control; acidity, one and five-tenths per cent.

The opalescence of the milk has always been attributed to the alkalinity, but the clearing is often slightly apparent even before the milk has reached the neutral point to phenolphthalein, so that it becomes a question whether it may not be due, partially at any rate, to production of casease by the bacilli.

Neither the intermediates nor typhoid will ferment lactose even to the extent of acid formation, but acidity, as first shown by Theobald Smith, cannot be produced except from a fermentable carbo-hydrate. Both typhoid and the intermediates can ferment glucose and other mono-saccharids, the former without and the latter with production of gas.

Typhoid produces CO_2 in glucose media, but not more than can be absorbed, so that there are no visible gas bubbles. In speaking of gas formation in this paper only visible gas formation will be indicated.

Theobald Smith, therefore, concluded that there must be a second sugar, probably glucose or galactose, present in milk, to the fermentation of which the permanent acidity of typhoid and the initial acidity of the intermediates are due.

It was thought, therefore, that if this second sugar could be previously got rid of the opalescence might appear earlier, and to effect this sterilized milk in bulk was inoculated with typhoid and incubated for three days. Then boiled, brought to its initial reaction, tubed, and after sterilization inoculated with the various organisms. The opalescence under these conditions was noticeable somewhat earlier, but there was no marked advantage in adopting this method.

LITMUS MILK.

Experiments with litmus milk are practically repetitions of those with plain milk, but the color enables one to judge at a glance if the culture is more or less alkaline than the controls.

Besides this, in cases where there is alkali production the litmus milk turns blue long before there is any visible opalescence in plain milk, so that there is a considerable saving in time in making a diagnosis.

It was found that after an initial slight acidity the milk would appear slightly more blue than the controls in about five days with Hog cholera, Typhi murium, Gärtner, Hatton, Strong, Malcomb, Libman's X, Seemann, Kurth, and the blue color would continue to increase in intensity; the average at the end of ten days being about five-tenths per cent to phenolphthalein.

Bacillus O is somewhat erratic. It will sometimes turn the milk blue almost as quickly as the above, and at other times will remain acid for a considerable time.

The following remain acid for ten days: Gwyn, Badach, Milewsky, Noonan, Müller, and Case 7 — the final acidity being about one per cent.

On previously treating litmus milk with typhoid there is only a trace of initial acidity with any of the intermediates.

In Two Days.

(*a.*) Slightly more blue than control: Hog cholera, Typhi murium, Gärtner, Hatton, Strong, Malcomb, Libman's X, Seemann, Kurth.

(*b.*) Like controls: Bacillus O.

(*c.*) Faintly acid: Gwyn, Badach, Milewsky, Noonan, Müller, Case 7.

In Ten Days.

(*a.*) Very blue: Hog cholera, Typhi murium, Gärtner, Hatton, Strong, Malcomb, Libman's X, Seeman, and Kurth. Average alkalinity, five-tenths per cent to phenolphthalein.

(*b.*) Somewhat less blue: Bacillus O. Neutral.

(*c.*) Like controls: Gwyn, Badach, Milewsky, Noonan, Muller, and Case 7. Average acidity one per cent.

Controls; acidity, one and five-tenths per cent.

The previous treatment with typhoid enables the differences to be observed much earlier, but there is no saving in time unless the medium is ready for use. It might, how-

ever, be found advantageous to prepare all one's stock litmus milk free of the second sugar in this way, so as to have it ready for an emergency. Alkali production could then be much more quickly noted at any time than with litmus milk not so treated, and this would no doubt hold good for many bacteria other than the intermediates.

Petruschky's Litmus Whey.

Durham places great reliance on this medium as a means of differentiating between the various colon groups including B. lactis aerogenes and B. typhosus, but it must be confessed that New York whey failed to afford the same results as he obtained with the English variety. He found that B. coli communis clouds the whey, and makes it permanently acid. The Gärtner group cloud it, but after an initial acidity there is in four days a production of alkalinity which goes on increasing, whilst with typhoid the whey remains clear but becomes permanently slightly acid. Bacillus O and B. Gwyn which he distinguishes from the Gärtner group behave in litmus whey very similarly to B. typhosus, in that there is no subsequent production of alkali and very slight cloudiness.

Schottmüller found ten days to elapse before there was sufficient alkalinity for the blue color to be regained, and as with milk his Barg and Müller differed from the other four; producing permanent acidity so that they were not distinguishable from typhoid. These observations could not be confirmed in their entirety. It is true that those intermediates which cause milk to become opalescent in ten to twenty days cloud the whey much more than the others. Bacillus O appearing to hold an intermediate position in this respect, but the acidity remained unchanged in all of them for three weeks, so that the only difference was in the degree of clouding, — a rather uncertain factor on which to depend for diagnosis.

If, however, the whey is first treated with typhoid as already described for milk in order to get rid of the second sugar, much more positive results can be obtained. In this case B. coli communis causes marked cloudiness and perma-

nent acidity, whilst typhoid cultures remain clear and form no acid.

There is initial acidity with all the intermediates, but in four or five days they begin to show differences:

(*a.*) Cloudy and acid: Hog cholera and Seemann.

(*b.*) Cloudy and slightly alkaline: Typhi murium, Gärtner, Hatton, Strong, Malcomb, Libman's X, and Kurth.

(*c.*) Slightly cloudy and alkaline: O.

(*d.*) Slightly cloudy and acid: Gwyn, Badach, Milewsky, Noonan, Müller, and Case 7,

	In three weeks. Aver. alk.
Group (*a*) become alkaline in two to three weeks,	.1 per cent.
Group (*b*) become rapidly more alkaline	1 per cent.
Group (*c*) becomes more alkaline	.3 per cent.
	Aver. acid.
Group (*d*) acidity permanent	2.2 per cent.
Controls	.5 per cent.

It is rather puzzling to account for the much greater initial acidity in litmus whey than in litmus milk when first treated with typhoid, but presumably typhoid is able to grow so sparingly in the former that it is unable to reduce all of the second sugar, which must here be somewhat more concentrated than in the original milk. In any case, however, so far as differentiating between the intermediates is concerned, litmus whey seems to be less valuable than litmus milk. Not only is it much more troublesome to prepare, but the alkali production is not noticeable so early as with the milk, even when it is previously treated with typhoid.

On reviewing the experiments with milk and whey we find that the intermediates fall into two distinct groups, Bacillus O appearing to hold a somewhat intermediate position.

The table then may be rearranged in accordance with the groups. Malcomb being put first since it forms indol freely and, therefore, appears somewhat nearer to B. coli communis than the others.

Group I.	Case.	Intermediate.	Case.	Group II.	Case.
1. Malcomb	Irregular.	10. Cushing's O.	Typhoidal.	11. Gwyn ...	Typhoidal.
2. Strong	"			12. Badach ..	"
3. Hog cholera ...				13. Milewsky.	"
4. Typhi murium .				14. Noonan ..	"
5. Gärtner	Meat poisoning.			15. Müller ...	"
				16. Case 7....	"
6. Hatton	Meat poisoning.				
7. Libman's X....	Typhoidal.				
8. Kurth	"				
9. Seemann	"				

GAS PRODUCTION.

It is generally recognized that the intermediates can be distinguished from typhoid by their power of fermenting the disaccharid maltose and all the monosaccharids with formation of visible gas. On the other hand they can be distinguished from B. coli communis by their inability to form acid or gas in lactose media, but Durham and Cushing, so far as I am aware, are the only ones who have published observations on differences in gas production among the intermediates themselves. Durham found that Gärtner and its allies would form gas from glucose and other monosaccharids in Dunham's peptone solution, but that Bacillus O and B. Gwyn would not. Dr. Hewlett, of the New York Hospital, informs me that he could not confirm this observation of Durham's, but finds that all the intermediates he has tested will form gas under these conditions.

Cushing remarked that Bacillus O and B. Gwyn in dextrose bouillon produced gas more slowly than the other intermediates — only about half as much in the first twenty-four hours — and the same was the case with mannite so far as Bacillus O was concerned.

In the following experiments it was found that all the intermediates would ferment maltose, glucose, galactose, fructose, mannite, dextrin, and yeast broth with production of gas, but not lactose.

Inulin, gum arabic, and calcium lactate were also tried with negative results.

Where peptone bouillon was used as a basis it was prepared sugar free by Theobald Smith's method, and after testing with B. Coli Communis one per cent of the sugar or other material was added.

Bent tubes of which the closed arm is one hundred millimeters in length were used, so that the measurements given in millimeters of the gas formed will afford an idea of the relative amounts.

The amount of carbon dioxid formed was tested in many instances by lime water, but this was not found to assist in the classification to any extent. As a general rule, however, there appeared to be a rather larger percentage in the cases where fermentation was rapid than where it was slow.

It was not thought worth while to test the percentage of acid or alkali formed. Occasional rough tests with litmus afforded no definite results, and it did not seem as if accurate observations would lead to anything.

GAS PRODUCTION.

SUGAR FREE BOUILLON.

Glucose, one per cent.

Fermentation completed		Average gas in mm. 1st day.	2d day.	3d day.
1 day	Coli Communis, Strong	35	40	—
2 days	Malcomb, Hog cholera, Typhi murium, Gärtner, Hatton, Libman's X, Kurth, Seemann	30	35	
	Highest first day, 30 mm. Lowest, 24 mm.			
3 days	O, Gwyn, Badach, Milewsky, Noonan, Müller, and Case 7	10	24	27
	Highest first day, 22 mm. Lowest, 10 mm.			

Maltose, one per cent.

2 days	Coli Communis	21	30	—
	O, Gwyn, Badach, Milewsky, Noonan, Müller, Case 7	12	14	—
	Strong, Hog cholera	14	17	—
3 days	Malcomb, Typhi murium, Gärtner, Hatton, Libman's X, Kurth, Seemann.	15	22	26

GAS PRODUCTION.

PEPTONE SOLUTION.

Gas in mm. per 5 mm.

Fermentation completed in		Average gas in mm. 1st day.	2d day.	3d day.
2 days	Coli Communis	20	28	—
	Malcomb, Strong, Hog cholera, Typhi murium, Gärtner, Hatton, Libman's X, Kurth, Seemann	15	25	—
	O, Gwyn, Badach, Milewski, Nooman, Müller, and Case 7	Bubble		
	Malcomb, on 5 mm.			
2 days	Coli Communis	18	27	—
	Malcomb, Strong, Hog cholera, Typhi murium, Gärtner, Hatton, Libman's X, Kurth, Seemann	16	29	—
	Hatton, Malcomb, and Kurth formed a little more by third day.			
	Highest on second day, 33 mm. Lowest, 17 mm.			
	O, Gwyn, Badach, Milewski, Nooman, Müller, and Case 7	8	11	
	Highest on second day, 13 mm. Lowest, 9 mm.			

GAS PRODUCTION.

SUGAR FREE BOUILLON.

Galactose, one per cent.

Fermentation complete in		Average gas in mm. 1st day.	2d day.	3d day.
1 day	Coli Communis	37	—	—
2 days	Malcomb, Strong, Hog cholera, Typhi murium, Gärtner, Hatton, Libman's X, Kurth, and Seemann	20	34	—
3 days	O, Gwyn, Badach, Milewski, Noonan, Müller, and Case 7	6	18	23
	Fructose, one per cent.			
1 day	Coli Communis	40	—	—
2 days	Malcomb, Strong, Gärtner, Hog cholera, Typhi murium, Hatton, Kurth, and Seemann	25	35	—
3 days	O, Gwyn, Badach, Milewski, Noonan, Müller, and Case 7	10	27	Trace more.

GAS PRODUCTION.

SUGAR FREE BOUILLON.

Mannite.

Fermentation complete in		Average gas in mm. 1st day.	2d day.	3d day.
2 days	Coli Communis	67	77	—
	Malcomb, Strong, Typhi murium, Gärtner, Hatton, Kurth, Seemann,	38	48	-
3 days	Hog cholera, Libman's X	25	37	43
	First day, highest, 55 mm. Lowest, 24 mm.			
2 days	Badach, Müller	10	30	
3 days	O, Gwyn, Milewsky, Noonan, Case 7	13	23	28
	First day, highest, 19 mm. Lowest 10 mm.			

The tables are compiled from two complete trials of the whole series in different batches of bouillon; other trials, each less complete in itself, afforded similar results.

Gas Production.

Yeast broth prepared by Spronck's method is uncertain. One batch failed to give any gas formation at all with any of the intermediates or B. coli communis, but as a rule there is a slight production of gas, more noticeable — about twelve millimeters — with Group II. than with Group I., — about eight millimeters, — and least of all with B. coli communis, which shows little more than a bubble.

B. coli communis and Group I. produce no further gas after the first twenty-four hours, but Group II. generally goes on fermenting for three days. But yeast broth is troublesome to prepare, and after the failure of one entire batch it was not tried again, since at the best it is inferior to glucose or mannite for differentiation. But the fact that B. coli communis in yeast broth forms less gas than any of the intermediates seems to indicate that the fermentable carbohydrate cannot be a sugar. Since dextrine would very likely be present in yeast, one per cent solutions in sugar free bouillon were tried with results very similar to those obtained by yeast broth; somewhat more gas, however, being formed by all the organisms. An experiment was made by growing B. coli communis in one per cent dextrine in bulk. After sterilizing and neutralizing it was put into fermentation tubes and inoculated with intermediates, but no gas was produced. It may be worth while to follow up this question of gas production in yeast broth and dextrin solutions at some future time, but it is doubtful if much can be learnt from the latter. The term dextrin, as proved by Brown and Morris, covers a multitude of inversion products intermediate between starch and maltose, and it is not likely that these would ever be present in the same proportions in the various commercial dextrins.

On reviewing the tables we find that there is distinctly less gas production by Group II., including Bacillus O, than by

Group I., and that the action by the former is usually somewhat slower.

The differences are best brought out by glucose, Group II. taking three days to complete the fermentation in bouillon, and producing only half as much gas as Group I., the highest quantity of gas with II. always falling below the lowest with I. Glucose in peptone solution is even better for differentiation, the mere bubble of gas formed by Group II. almost coinciding with Durham's observation that Bacillus O and B. Gwyn form no gas in this medium.

Photographs 3 and 4 show the progress of gas formation in glucose media, the marks indicating the gas formation day by day. Where the level of the fluid stands a little higher than the lowest mark this is due to contraction of the gas on cooling down after being taken out of the incubator.

Mannite also differentiates well, the highest of Group II. in twenty-four hours being five millimeters below the lowest of Group I.

With maltose, galactose, and fructose the fluctuations are greater, so that the differences between certain individuals of each group are not so great as the averages would seem to indicate.

COLORED MEDIA.

Rothberger in 1898 first advocated the use of neutral red agar for differentiation between B. coli communis and B. typhosus, the latter having no action on the color, whilst the former in twenty-four hours reduces it to a bright yellow with a marked greenish fluorescence. Since then neutral red agar has come into very general use, Makgill and Savage (1901) recommending it very strongly as a means of testing for the presence of B. coli communis in water.

Schottmüller tried it with his cultures and found that they reacted like B. coli communis, the color being changed to yellow in twenty-four to forty-eight hours.

The formula is:

Neutral plain agar	100 cubic centimeters.
Sat. aq. sol. of neutral red	1 cubic centimeter.
Glucose	.3 grams.

About eight to ten cubic centimeters are put into each tube and either deep stick or shake cultures made, the latter affording more uniform results. Very striking effects were obtained with this medium.

B. coli communis changes the color more rapidly than any of the intermediates, the yellow showing throughout the tube in twenty-four hours. With Group I. of the intermediates there is still, usually though not invariably, a narrow band of red at the surface in twenty-four hours, but in forty-eight hours the medium is entirely yellow. With Group II. there is no change in twenty-four hours except partially in the case of Bacillus O, but in forty-eight hours to three days the medium becomes yellow except for a depth of about two to five millimeters, at the surface. In both groups as with B. coli communis there is a considerable evolution of gas in twenty-four hours. B. typhosus and B. dysenteriæ form no gas and the color remains permanently unchanged.

After four or five days the red color begins to return from above downwards with all the members of Group II., and gradually spreads until in three weeks or sometimes less the medium appears red again throughout, whilst with Group I. the yellow color is permanent. The only exceptions to this rule are Seemann and Bacillus O. About once in three or four trials the former would regain color though slowly, and the latter would not, so that these two appear to hold an intermediate position between the two groups so far as the reaction is concerned. None of the others ever failed to fall into their own group.

The colored plate serves as an illustration.

Neutral red, therefore, does not differentiate very clearly between B. coli communis and Group I., but is probably the most valuable medium we have for distinguishing the two groups among the intermediates themselves. No mention of this subsequent return of the color could be found in any of the published accounts, nor can any explanation of the reason for it be offered.

Toward sodium sulph-indigate the intermediates react in the same manner, and the return of the blue-green color in

Group II. is even more rapid than with neutral red. But the color fades naturally even in the controls after eight to ten days, so that this dye will probably not prove to be reliable.

Other colored media were also tried and somewhat similar results were obtained with saffranin and methylene blue, but the reaction seems more uncertain. Fuchsin, night blue, Bismarck brown, and some other anilin dyes were also tried but with indifferent success.

Fermentation and colored media tests therefore show that the intermediates group themselves in the same manner as when tried with milk and whey, B. Seemann and Bacillus O appearing to be a little undecided as to which group they belong to. On the whole, however, B. Seemann appears to be more closely allied to Group I., and Bacillus O to Group II., so that they may be assigned to these respectively.

Culturally it is evident that there are two distinct groups among the intermediates, and of these Group I. appears, especially in regard to gas production and behavior in colored media, to stand nearer to B. coli communis than Group II. It is therefore proposed to call the groups paracolons and paratyphoids respectively, these terms appearing the more suitable in that, so far as is known at present, the members of Group II. cause no infections in man other than typhoidal.

It now remains to determine if these are groups in the sense that the various strains of B. coli communis form a group, or constitute two distinct species in the sense that the typhoid bacillus is a distinct species. Agglutination tests alone can help us to decide this question. Since the paratyphoids are so much alike in their pathogenic properties, it seems probable that they are a species rather than a group.

AGGLUTINATION.

A few of the published observations may be summarized as an introduction to the subject.

To begin with, Achard and Bensaude (1896), isolated two paratyphoid bacilli which were clumped by serum of the

patients, and slightly by typhoid serum. Three cultures of Nocard's B. psittacosis from different sources were clumped by the serum.

Widal and Nobécourt (1897) obtained a paracolon bacillus from a thyroid abscess, which was clumped (one to one thousand) by the patient's serum, but the serum was practically inactive (one to one hundred) on a whole series of allied forms, colons, paracolons, including B. psittacosis, and B. typhosus. Cushing (1900) found that serum of the patient from which his Bacillus O was isolated clumped it strongly up to one to eight thousand, but was negative to B. Gwyn, B. coli communis, and B. typhosus. Later the blood of a rabbit inoculated with Bacillus O clumped this bacillus and showed slight reaction to the bacillus of hog cholera. The latter was then injected, with the result that the rabbit's serum was active to Bacillus O, and the bacillus of hog cholera (one to five thousand), but practically negative to all others of the group including B. Gwyn.

Durham prepared a rabbit's serum with B. Gwyn which clumped it (one to twenty thousand), but had not the slightest effect upon Bacillus O (one to one hundred), whilst with serums prepared from the Gärtner group he found but few that would interact. He concluded, therefore, that the clumping test has only a limited value in the diagnosis of species.

Schottmüller observed that his B. Seemann and three others, which culturally belong to our Group I., would interact but slightly with Barg and Müller, Group II., but among themselves much more decidedly.

Kurth's two bacilli interacted, and were also clumped by the serum of three other typhoidal cases in which the Widal reaction was negative with typhoid.

It did not then appear on reviewing the literature as if the agglutination test would be of much value for classification purposes, but in this expectation I was agreeably disappointed, for a series of experiments on rabbits only served to accentuate the probability of Group II. constituting a distinct species and not merely a group.

A few preliminary remarks on the methods adopted may be inserted.

A number of rabbits were immunized to various organisms by subcutaneous injections of living agar cultures, beginning with one-twentieth or one-tenth and gradually increasing the amounts until a whole culture could be used without harmful results. Beyond this it was unnecessary to go, as the object was, not to get very highly potent serums, but to have them all as nearly as possible equally active towards their own organisms. The injections were made every week or ten days.

To take blood, the ear was shaved, dried, and the marginal vein pricked with a needle. A bulb pipette, as used for testing the alkalinity of the blood, containing five cubic centimeters and marked at twenty-five one-thousandths cubic centimeters on the stem, was found a convenient instrument; the blood being drawn up to twenty-five one-thousandths cubic centimeters and the bulb then filled with sterilized water.

This made the dilution one to two hundred and from this further dilutions could be made. The pipette was thoroughly washed out between each operation with distilled water, alcohol, and ether, but special aseptic precautions were not necessary, since the serum was always tested very shortly after being drawn.

It was found, however, that it could be kept perfectly well for two weeks in cold storage without losing its efficiency.

To test the reactions a number of small test tubes ($3'' \times 1/2''$) were taken and hung up side by side in a home-made wire rack. With a pipette one cubic centimeter of diluted blood was run into each of the tubes, and then one cubic centimeter of the culture to be tested; broth cultures not over twenty-four hours old being used for the purpose. The lowest dilution then for regular routine work was one to four hundred.

In looking over tables of reactions it is very confusing to find for example that one bacillus is acted on at one to ten thousand, another at one to two thousand, and a third at one to two hundred and so on. Such tables unless they are

closely studied are of no value in giving an idea of mutual reactions. It was thought therefore simpler and more practical to take a definite standard for interactions, and this was fixed, arbitrarily it must be confessed, at one to four hundred in four hours. It may be mentioned, however, that when there were mutual reactions at one to four hundred, these practically always occurred also at one to four thousand, in cases where the rabbit's serum agglutinated its own organism up to one to ten or twenty thousand.

Readings were taken in one, two, four, and twenty-four hours, and as soon as flakiness was visible to the eye this was marked as +. After the clumps had all fallen to the bottom of the tubes and the liquid above appeared perfectly clear, a + + sign was used as an indicator.

(See Plate XVI. Fig. 4.)

Hanging drops were frequently made as controls, but where the action is closely watched it is not necessary to do this, except occasionally so as to make sure that one is not falling into error. It not infrequently happens, however, that the bacilli will gradually sink to the bottom in a cloud, without any previous visible flakiness, leaving the liquid above quite clear. If this process be watched it can be easily distinguished macroscopically from true reaction, but if the final stage alone be observed mistakes may easily arise unless a hanging drop be made, in which case only small atypical clumps can be seen, the majority of the bacilli being free and actively motile. It was found that typhoid more especially is liable to show this pseudo-reaction.

SERUM 1–400 IN 4 HOURS.

		1. Malcomb.	2. Strong.	3. Gartner.	4. Kurth.	5. Seemann.	6. O.	7. Gwyn.	8. Case 7.	9. 7 + Typhoid.	10. O. + K.	11. 7 + Typhoid + Hatton.	
	1. Coli	−	−	−	−	−	−	−	−	−	−	−	Coli.
	2. Malcomb	+	−	−	−	−	−		−	−	−	−	Mal.
	3. Strong	++	++	++	++	++	++		++	++	++	++	Str.
	4. Hog Cholera	−	−	−	−	−	−	−	−	−	?+	−	Hog.
	5. Typhi Murium	−	−	−	−	−	−	−	−	−	−	−	Mur.
Group I.	6. Gärtner	−	−	++	−	−	−	−	−	−	−	−	Gart.
	7. Hatton	−	−	−	−	−	−		−	−	−	++	Hatt.
	8. Libman's X	−	−	−	++	++	−	−	−	−	++	−	X.
	9. Seemann	−	−	−	++	++	−	−	−	−	++	−	Seem.
	10. Kurth	−	−	−	++	++	−	−	−	−	++	−	K.
	11. O	−	−	−	−	−	++	++	++	++	++	++	O.
	12. Gwyn	−	−	−	−	−	++	++	++	++	++	++	Gwyn.
	13. Badach	−	−		−	−	++		++	++	++	++	Bad.
Group II.	14. Milewsky	−	−	−	−	−	++	++	++	++	+	+	Mil.
	15. Noonan	−	−	−	−	−	++	++	++	++	+	+	Noon.
	16. Müller	−	−	−	−	−	++	++	++	++	+	++	Müll.
	17. Case 7	−	−	−	−	−	++	++	++	++	++	++	7.
	18. Typhoid	−	−	−	−	−	−	−	−	++	−	++	Typh.

The accompanying table is given as an example of the results obtained. The Gwyn rabbit unfortunately died before its serum was tested with all of the cultures. Eleven serums were used in all, over five hundred tests being made at the standard dilution besides a large number of others. No. 9, called 7 + Typhoid, was prepared by injections of equal quantities of Case 7 and typhoid, and was found to agglutinate all of Group II. and typhoid. Later on these were discontinued and Hatton used for injections. The serum No. 11 then agglutinated Hatton in addition to the others. After the Bacillus O serum had been thoroughly tested, injections of Bacillus O were left off and X used instead. The serum No. 10 then agglutinated X, Kurth, and Seemann without losing its activity towards Group II. The results obtained were remarkably constant, the only exception being that the Bacillus O rabbit after inoculation with Bacillus O for two months and then one month with X was found to agglutinate the Bacillus of hog cholera, though slowly and somewhat imperfectly.

Cushing, as has already been remarked, observed that the Bacillus of hog cholera reacted slightly with the serum of his Bacillus O rabbit. Although it has not been mentioned before, it was found in the course of these experiments that the Bacillus of Hog cholera has a tendency to form rather less gas and alkali than other members of Group I., so that it may have some affinities with Group II. Still the evidences in avor of this are so slight that they will not be taken into consideration in classifying.

It will be noticed from the table that B. Strong reacted readily with all of the serums.

This led to its being tested with normal rabbit's serum, by which it was agglutinated just as quickly, even up to one to four thousand. It was also found to be agglutinated one to four hundred by normal serum of man, and guinea-pig; but not by that of a monkey or dog. No explanation can be given for this extraordinary fact.

Nor can any explanation be offered for the failure by Cushing and Durham to get interactions between Bacillus O

and B. Gwyn. There was no question about it at all in my experiments, and, in doubt at one time as to whether mine was really a Bacillus O culture, I wrote to Dr. Johnston of the Johns Hopkins laboratory, who kindly sent me another, which, however, behaved culturally and in its agglutinating properties precisely like the first.

Dr. Johnston writes that in his own experience Bacillus O interacts imperfectly with other members of Group II. Dr. Hewlett, however, finds that Bacillus O is agglutinated by B. Noonan serum.

On examining the table we find that all the members of the paratyphoid group interact freely, quite as readily in fact as various strains of typhoid would be likely to do. It appears therefore that we must here be dealing with a distinct species, the members of which are indistinguishable from each other either culturally or by agglutination tests, and can cause a typhoid-like infection in man.

Turning to the paracolons, however, we find that the two which are not pathogenic for man, the bacillus of hog cholera and B. typhi murium, do not react with any of the serums prepared, with the doubtful exception already mentioned, nor do the serums of Strong and Malcomb take effect on any but their own organisms. Neither of these, however, caused meat poisoning or typhoidal symptoms in man.

The meat-poisoning group, of which only two, B. Gärtner and Hatton, were tested, is a very large one and the number of bacilli known to belong to it has been constantly increasing since Gärtner first isolated his in 1888. It is not worth while to refer individually to the numerous published accounts of cases where the bacilli have been isolated, but they all tend to show that the members of the group are culturally similar, and belong to our Paracolon group. But when tested for their agglutinating properties it becomes evident that there are differences between them.

The observations of Durham and Fischer may be briefly summarised.

The former found that the races Gärtner and Moorseele were strongly affected by Gärtner serum, whilst the races

Hatton, Basenau, Gand, Günther, Sheffield,* and some others were little or not at all affected.

Bernard Fischer, who isolated bacilli from meat-poisoning cases in the towns of Haustädt and Rumfleth, near Kiel, observed that they showed mutual reactions and interacted also with Gärtner and Van Ermenghem, slightly with Kaensche and Günther, but not at all with Basenau, Grünthal or Glückstadt.*

On consulting the accompanying tables it will be seen that B. Gärtner and Hatton did not interact.

Enough then has been done to show clearly that taken all together the bacilli causing "meat-poisoning" epidemics are not a distinct species within the paracolon group, but simply members of that group; a group made up of various races just as is the case with the B. coli communis group.

From among the paracolons tabulated here, however, we can pick out three which do show mutual reactions and, unlike the "meat poisoners," appear to constitute a distinct species within the group: namely, Libman's X, Kurth, and Seemann, and it is precisely these and these only which were isolated from cases resembling typhoid fever, Malcomb and Strong having been ruled out.

It is interesting to observe that they came from New York, Bremen, and Hamburg respectively.

It would be necessary to compare a larger number of these before any definite conclusion can be reached as to whether they constitute a distinct species or not, but they may provisionally be put in a separate sub-group.

According to their cultural properties they should belong to the paracolons, but since they cause typhoidal symptoms, and are here assumed to be a distinct species, it seems better to class them with the paratyphoids, distinguishing between them and the first mentioned species.

The following classification is therefore suggested:

Paracolons.— Those which do not cause typhoidal symptoms in man. A group containing numerous different members, but culturally alike.

* These names represent various strains of the meat-poisoning group.

Paratyphoids.— Those which cause typhoidal symptoms.

α A distinct species culturally unlike the paracolons.

β A distinct species culturally resembling the paracolons.

The question then arises whether in cases of suspected typhoid which are negative to Widal with typhoid cultures it is likely to prove advantageous to try the serum with other organisms.

There seems little doubt that a test with an *α* paratyphoid may in some cases be of assistance in diagnosis, and it is quite possible that the *β* paratyphoids may prove useful in some instances. If the above classification is found on further investigation to hold good, it will certainly simplify matters for the clinical pathologist, since he will be able to make tests with intermediates in a systematic manner instead of blindly proceeding with any culture he may have in stock in cases where his typhoid cultures fail him.

Since finishing the above Dr. Longcope of Philadelphia has kindly sent me, with permission to mention them, cultures of intermediates obtained by him from the blood of two patients with typhoidal symptoms at the Pennsylvania Hospital. One of these, Case 4799, belongs clearly, both in its cultural and agglutinating properties, to the *α* paratyphoids, whilst the other, Case 5207, culturally resembles the paracolons and *β* paratyphoids, but is not agglutinated by any of the serums.

All further work done on the *α* paratyphoids has only helped to confirm the opinion that they are a distinct species, but on immunizing more highly the *β* paratyphoid rabbits, Seemann serum has developed the power of agglutinating the bacillus of hog cholera, B. typhi murium, and B. Hatton, though somewhat imperfectly. Kurth and O + X, however, continue to act as already described. This observation and the fact that Dr. Longcope's 5207 is not affected by any of the serums tend to render doubtful the likelihood of our being able to recognize a *β* paratyphoid species; but I shall hope to return to this work later on and endeavor to

answer some of the unsolved problems afforded by the alkali producers.

Strong has just published his case in the May number of the "Johns Hopkins Bulletin." It appears to have resembled typhoid clinically, but the author leaves it an open question as to whether the bacillus isolated from the spleen after death was actually the cause of the infection or not.

NOTE. — In the Münchener Medizinische Wochenschrift for April 15, 1902, has appeared a description by Brion and Kayser of Strassburg of an intermediate bacillus isolated from the blood of a patient with typhoidal symptoms in October, 1901. The serum of the patient clumps this bacillus, and also Schottmüller's Müller, but not Seemann, whilst the bacillus itself is not an alkali producer, and forms gas more slowly than B. coli communis. It appears then to be an *a* paratyphoid, and this, including Schottmüller's Barg, brings the number of those described within the last three years up to ten, eight of which have been under observation here.

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DESCRIPTION OF PLATE XV.

FIG. 1. Cultures in milk tubes. Three weeks old. Left, Group II. Center, Cushing's Bacillus O. Right, Group I.

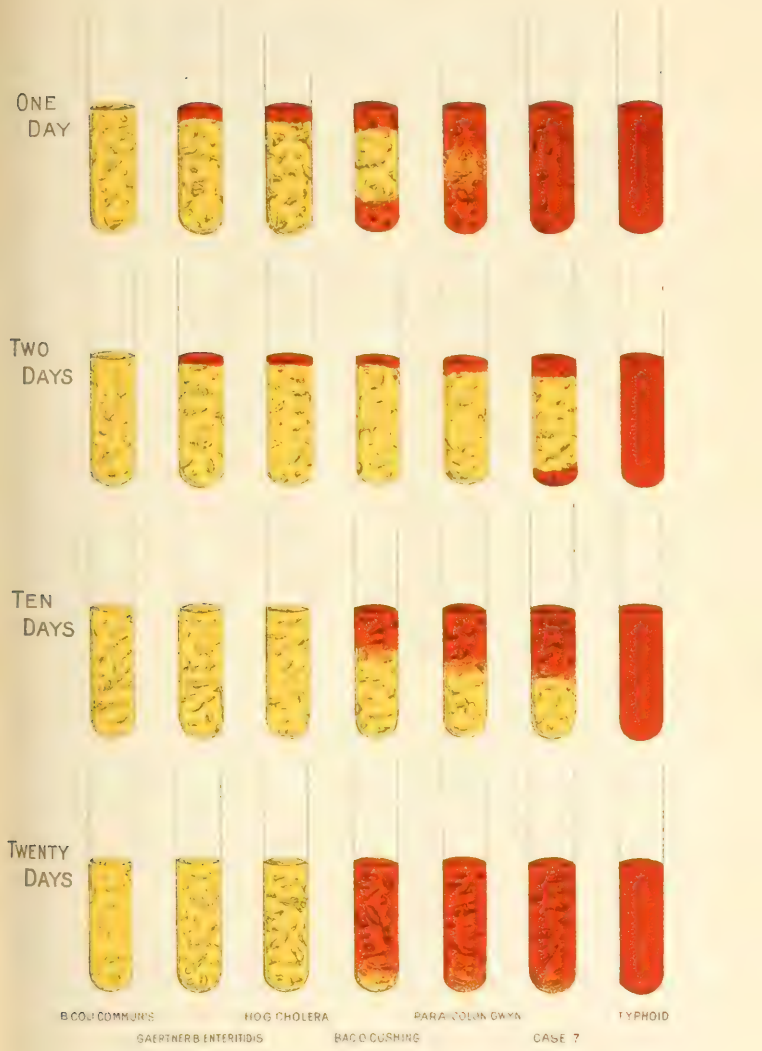
FIG. 2. One per cent glucose in sugar free bouillon. Left, B. coli communis. Center, Group I. Right, Group II.

PLATE XVI.

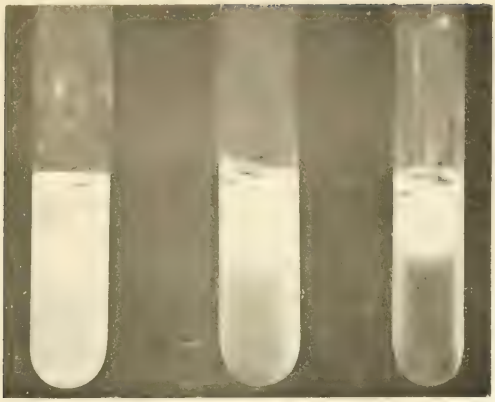
FIG. 3. One per cent glucose in peptone solution. Left, B. coli communis. Center, Group I. Right, Group II.

FIG. 4. Agglutination tubes. Left, no agglutination. Center, some agglutination. Right, marked agglutination.

NEUTRAL-RED AGAR



BUXTON. PARACOLON GROUP.



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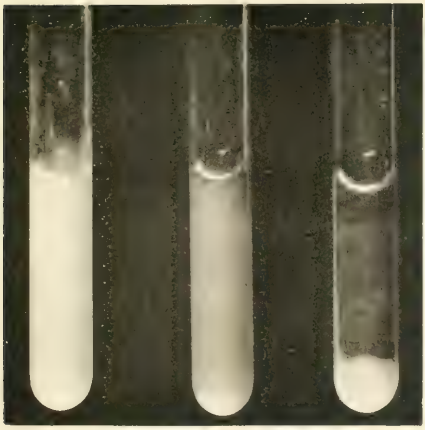

B

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BACTERIAL PURIFICATION OF SEWAGE.

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In this country of enormous lakes and rivers, and comparatively great distances between the larger towns, the necessity for purification of sewage before its final discharge into the water courses has so far hardly been felt. It is only a matter of time, however, before something must be done, and the rising generation should begin to gain some knowledge of the question, since it will surely become a burning one before their race is run.

Experiments have been carried on during the last 14 years at Lawrence, Mass., under the auspices of the State Board of Health, but England from force of necessity has been the birth place of most inventions for solving the problem. So long as 40 or 50 years ago the pollution of rivers had reached such a point that it was clear this state of things could not be allowed to continue, and in 1876 the Rivers Pollution Act was passed, under which all towns were enjoined from running crude sewage into the water courses. In consequence of this act a host of purification schemes arose, all of them more or less inefficient.

In the first place sewage farms were started, but were found impracticable in most instances, owing to the impossibility of obtaining sufficient suitable land at reasonable rates. Numerous chemical purification methods were also tried, and ultimately lime, or lime mixed with ferrous or aluminum sulphate, was found to be the best precipitant; the insoluble matters being practically all carried down to form a sludge, whilst the effluent was clear and free from odor. This, however, by no means solved the difficulty, for the sludge proved costly to handle and practically valueless for manure, whilst the effluent was just as prone to putrefaction as the original sewage, since the organic substances in solution had not been broken down in the least.

By degrees, however, it came to be recognized that the destruction of excrementitious and other

dead organic matter as carried out in soil and water by nature is not a chemical but a purely biological process due to the action of bacteria, and about 1890 these ideas began to take a practical shape; suggestions being made to imitate nature and so improve on her methods that the purification of sewage could be carried on "under control" in a more concentrated way than provided by her.

It may be said here in a general way that this is effected by running the sewage through tanks and coke beds in which the bacteria grow and multiply, attacking the organic matter in the sewage and breaking it down into simpler nonputrescible compounds. It is not necessary to seed the tanks and beds with special bacteria, since there are countless numbers of the right kind always existing in the sewage, affording a constant fresh supply.

Before describing the various biological filtration processes in use at the present day, some preliminary remarks are necessary in order to gain a clear idea of the subject.

Sewage consists of water containing about one-tenth per cent. of mineral and one-tenth per cent. of organic matter. The mineral matter may be ignored, since it is not liable to putrefaction. Of the organic matter about one-half is insoluble, being held in suspension, and one-half is in solution, but the strength of course varies within wide limits. On account of the presence of organic matter sewage is a favorable nutrient medium for bacteria, many of which, in the process of development, evolve offensive odors. The object then is to induce the bacteria so to change the sewage as quickly as possible in an inoffensive way, that by the time it is finally discharged into the water courses, it no longer affords a favorable medium for their growth.

All bacteria require oxygen for their development. Some can only make use of free oxygen and oxidize, so are called obligate ærobics, whilst to others free oxygen acts as an antiseptic, such bacteria being only able to make use of oxygen stored up in organic matter which they deoxidize in the process. These are the obligate anærobics. The vast majority of bacteria, however, are facultative anærobics, i. e., they can make use of free oxygen or, in its absence, utilize stored oxygen, either in organic or inorganic combination, and act as oxidizers or deoxidizers,

according to the nature of their environment. We may call these facultative anærobics or facultatives.

Bacteria are only able effectively to break down insoluble organic matter in such a way as to make it soluble when they are acting anærobically. Oxidation will change it, though slowly, in such a way that it will be less liable to putrefaction, but a great part of it will still be insoluble and soon clog the filters.

Sewage, therefore, must be subjected to anærobic action at an early period of its purification in order to liquefy the insoluble matter and prepare it for oxidation.

Generally speaking, it may be said that bacteria acting anærobically evolve offensive volatile products—sulphur compounds and methylamines—whilst in ærobic conditions of growth their volatile and gaseous products are inoffensive because more completely broken down. The former may be called putrefaction and the latter decomposition.

The object then will be to subject the sewage to as little anærobic action as possible; only sufficient, in fact, to liquefy the solids, and then pass it on to be oxidized by ærobic action.

In tracing the processes by which sewage is purified it will be best to commence with the solids, since the organic matter already in solution is simply in stages which will be reached by the former during purification. There are two kinds of solids, the carbohydrates, such as starch and cellulose, and nitrogenous proteids. The final products of purification are gases, which, of course, escape, water, and inorganic nitrates, which remain in solution; the latter being of great importance since it is in the form of nitrates that nitrogen is utilized by higher chlorophylous plant life. Since the carbohydrates contain no nitrogen, it will be sufficient to say of them that they are liquefied (hydrolysed) by the anærobics, and the soluble products, either anærobically or ærobically, further broken down until they are entirely reduced to gases and water.

The disposition of the proteids needs closer attention. The first action is essentially hydrolytic—simplification with addition of water—the obligate anærobics secreting soluble ferments or enzymes, which digest the insoluble proteids, in the same

way as animals digest them by means of enzymes secreted by the mucous membrane of the intestinal tract, changing them to soluble peptones.

The peptones are then attacked by bacteria which break them down into gases and various soluble amido compounds; this being done either by oxidation or deoxidation, the products varying in nature according to the action to which they have been subjected. In deoxidation, for instance, the principal gas generated is CH_4, and in oxidation CO_2; the amido compounds probably differing to some extent. The term amido compounds is here used for the sake of brevity, in a much wider sense than is strictly speaking correct, being intended to cover any nitrogen containing compounds simpler than peptones, but more complex than inorganic ammonium salts.

The next step is one principally of oxidation, though it may also take place anærobically by deoxidation, the amido compounds being changed to CO_2 and NH_3; these combining together to form inorganic ammonium carbonate which remains in solution. Gases are also evolved during this process, but these need not be taken into consideration.

A highly specialized strictly ærobic group of organisms, the nitrosifiers, now come into play, by which the ammonium carbonate is oxidized to nitrous acid; the latter combining with any potassium, sodium, or calcium bases present and forming nitrites.

The nitrites are then still further oxidized to nitrates by means of the nitrifiers, another group quite distinct from the nitrosifiers. In speaking, however, of nitrification, this is taken to cover the action both of the nitrosifiers and the nitrifiers.

The bacteria of nitrification cannot develop in the presence of much fermentable organic matter, so do not grow on the ordinary gelatine or agar plates of the bacteriologist. Winogradsky succeeded in isolating them on plates containing a gelatinous silicate as the solid medium, and his assistant, Omeliansky, has more recently used gypsum for this purpose; the nutrient media in each instance being ammonium salts for the nitrosifiers and nitrites for the nitrifiers, with solution of which the solid materials are moistened.

The process may be tabulated as follows:

	Changed to	By	Action
Insoluble proteids.	Soluble Peptones	Enzymes secreted by anaerobics	Hydrolysis
Soluble peptones.	Unstable amido compounds	Anaerobics or facultatives	Deoxidation or oxidation
Amido compounds	Ammonium salts	Facultatives	Oxidation or deoxidation
Ammonium salts	Nitrites	Nitrosifiers	Oxidation
Nitrites	Nitrates	Nitrifiers	Oxidation

Besides the above mentioned products there is a small amount of an irreducible insoluble organic substance formed, called humus, but not sufficient to clog the filters to an appreciable extent. The presence of a little humus is advantageous since the nitrifying bacteria seem to be able to make use of this nonfermentable matter in their metabolism.

A few remarks by way of explanation may here be inserted.

1. In speaking of hydrolysis—simplification with addition of water—it must be understood that this action is not confined to liquefaction of solids. Many of the soluble compounds are rendered more simple in construction by hydrolysis; the conversion of urea into the more stable ammonium carbonate by the micrococcus ureæ may be given as an example.

2. *Apparent Oxidation.*—Even under anærobic conditions there may be apparent oxidation, i. e., the resulting simpler compounds may contain relatively more oxygen than the original. But this is not in reality an oxidation, but only a loss of C, H or N, which are evolved as gases.

3. *Loss of Nitrogen.*—In the breaking down of nitrogenous compounds by bacteria there is always a loss of nitrogen evolved as N or as NH_3 if there is not enough acid present for the latter to combine with as ammonium salts. Free N is evolved by the action of the anærobics, being released from its organic combinations as such, but many of the facultatives in the absence of free oxygen can attack the nitrates and nitrites already formed and utilize their O; thus reducing them to NH_3 or free N. Such a

process is called denitrification, and occurs very freely in sewage beds where there is no attempt to keep the facultatives and nitrifiers separate. As will be seen, the continuous filtration systems are the only ones in which a complete separation is approached, and with these there is comparatively little loss of nitrogen. In manure heaps there is considerable loss on account of this denitrification, detracting to a great extent from the value of the manure as a fertilizer, whilst in natural processes in the soil it also occurs, resulting in loss of nitrogen.

4. *Chemical Tests.*—In testing sewage and the purified effluent chemically one estimates:

(a) The nitrogen existing as ammonia in inorganic combination.

(b) The nitrogen existing as ammonia in organic combination—albuminoid ammonia.

(c) The nitrites and nitrates.

(d) The oxygen absorbed. By O absorbed is meant the amount of it extracted from an acidified solution of potassium permanganate acting at room temperature for four hours. This varies much according to the nature of the organic compounds; the carbohydrates acting most energetically. The test may therefore be taken as roughly indicating the amount of carbohydrates, although it is of value more as a comparative than an absolute test, since the more unstable amido and other compounds and even inorganic sulphides, products of deoxidation, are also quite active.

The usual standard for a purified effluent in England is a maximum of 1 per 100,000 for the albuminoid ammonia, and 1 per 100,000 for the O absorbed.

5. The incubator test is of comparatively recent origin, having been in use only 4 or 5 years, but for practical purposes is the most important of all, since it aims to determine whether the final effluents are prone to putrefaction or not. A sample of the effluent is submitted to the O absorbed test for 3 minutes and the amount noted. A quart bottle is then filled with another, but similar, sample of the effluent and kept well stoppered at 80° F. in an incubator. After 6 or 7 days it is tested for three minutes; the odor and appearance being also noted. If there is an increase in O absorbed, this is looked

upon as evidence of putrefaction, since it means that there is an increase of the more unstable organic compounds and inorganic sulphides in consequence of deoxidation, whilst no change or a decrease in the amount of O absorbed indicates that the organic matter has been broken down to such an extent that there is no longer sufficient pabulum present to permit of increase of bacteria under anærobic conditions. The anærobics always require a good, strong diet, and so do the facultatives in the absence of O, although when there is plenty of this they can get along with very little nourishment.

6. Bacteriological tests are also valuable. Crude sewage contains one to ten millions or more bacteria per cubic centimeter, as shown by the usual methods of plating out, and this number may be reduced to a few thousands or even hundreds in a good final effluent. The obligate anærobics and nitrifying organisms will not grow on ordinary plates, so this refers only to the facultatives which are to some extent oxidized out of existence during purification processes.

It may, however, be at once remarked that, although it is quite possible to produce an effluent which would pass as a good drinking water according to bacteriological standards, yet the expense of this on a large scale is prohibitive. In practice the number of bacteria is not very materially reduced. Houston, in experiments with London sewage, found that the bacillus coli communis slips through the filters in large numbers, and from this it may be inferred that the typhoid bacillus would do so, too, if it happened to be present in the sewage at any time. It may be laid down, therefore, as a general, though at present ideal, rule that the water from no river into which even purified sewage is run should be used for drinking purposes without previous efficient filtration.

In determining the progress of purification bacteriologically, it is probable that results are usually considerably underestimated. In the suspended matter of the crude sewage the bacteria hang together in colonies or zoöglea, and Mr. Dibdin informed me last summer that, if the sewage be thoroughly beaten up first, the plate cultivations will show a hundred times more colonies than usual, so that instead of one to ten millions the numbers

will be more like a hundred to a thousand millions per cc. In the final effluents the suspended solids are practically removed so that the individual bacteria are more easily separated and the recorded results probably fairly accurately represent the true state of affairs.

Sewage, as it enters the main sewers, is called "fresh," and contains solid organic matters in lumps, soluble organic substances, ammonium salts, nitrites, nitrates and dissolved O. During the next 6 or 8 hours, which may be taken as a fair average time for it to reach the outfall, it undergoes some changes. The lumps are broken up, mechanically and perhaps also to some extent by bacterial action, into finer particles, and the bacteria present begin to multiply. The action is mainly by the facultatives, the dissolved O being first used up, after which nitrates and nitrites are reduced by coli communis and other "denitrifiers" which have this reducing power. The sewage is now "stale," containing comparatively finely divided particles and showing an increase of inorganic ammonia formed by action on the soluble nitrogenous matter, whilst the nitrites, nitrates and dissolved O have disappeared. It is now all ready for true anærobic action, and after this has proceeded to some extent it is called "septic" sewage.

The preliminary anærobic action was not understood at first, the supposition being that the purifying from start to finish was an oxidation process. Under this impression Col. Waring, 1894, introduced his sand filters with forced æration at Newport and other places in the United States. The purification of the sewage was effective, but the solids had no chance to get liquefied, so the filters quickly became clogged and had to be cleaned at great expense. In experiments on these lines at Lawrence it was found that the more complete the æration, the worse was the clogging.

Dibdin, about the same time, introduced his bacteria or contact beds in London; tanks, 3 or 4 feet deep, filled with lumps of coke or burnt clay, into which the sewage was slowly poured, allowed to remain "in contact" with the coke surfaces for a while, and then slowly drained off; the idea being that by this means the filters would be thoroughly ærated without the necessity for forcing air through

them. The contact beds were much more successful than Waring's, the sole reason, though this was

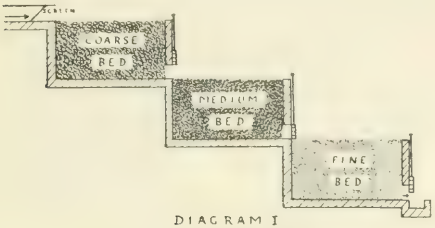

DIAGRAM I
TRIPLE CONTACT

not appreciated at that time, being that the aeration was less complete, so that some anærobic hydrolytic action was possible.

As soon as the double anærobic and aerobic action came to be recognized, attempts were made to keep each set of bacteria altogether separate so that the anærobics might do their work without interference by those which work best aerobically and vice versa.

The most successful methods are those of Cameron and Scott Moncrieff, 1896, the former introduced at Exeter and the latter at Caterham in England.

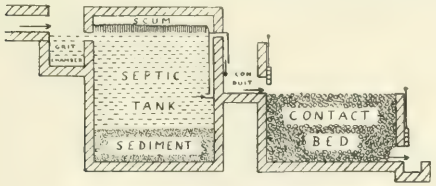

DIAGRAM II
SEPTIC TANK AND SINGLE CONTACT

Cameron (Diagram 2) allows the sewage to pass slowly and continuously through a closed wooden box—the septic tank—the effluent from which passes to a coke contact bed to be oxidized, the bed being alternately filled and drained. By means of this intermittent filtration aeration is accomplished since

air is drawn down through the coke in draining, as with the Dibdin beds.

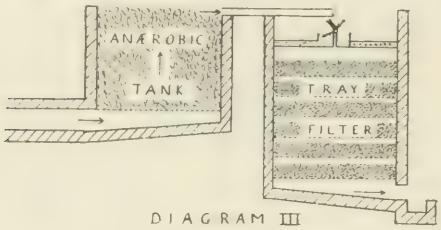

DIAGRAM III

SCOTT-MONCRIEFF SYSTEM

Scott Moncrieff (Diagram 3) constructs a tank containing broken flints or other coarse material resting on a false bottom, and allows sewage to flow in continuously from below, the action being altogether anærobic except to a limited extent at the surface. From the anærobic tank the sewage is passed on to filter through coke contained in a series of trays where the oxidizing action takes place; the flow being continuous and the fluid aerated in dropping from one tray to the next. By this and other somewhat similar methods of continuous filtration there is a better chance than in the contact bed for the facultatives and nitrifiers to get in their best work. The facultatives multiply mostly in the upper layers of the filter, reducing the soluble organic matter to ammonia. Below these comes a zone occupied chiefly by the nitrosifiers, and in the lower layers the nitrifiers predominate. The result is a purer, more highly nitrated effluent than is possible with the contact beds where there is no chance for the facultatives and nitrifiers to work independently of each other, but the general consensus of opinion is that the contact beds afford an effluent which is good enough for all practical purposes, and are cheaper to work on a large scale than any method of continuous filtration.

The processes may be tabulated:

		Method	Flow	Action
1.	Waring	Forced aeration through sand	Continuous	Entirely aerobic
2.	Dibdin	Coke beds Natural aeration by filling and draining	Intermittent	Anaerobic and aerobic alternately in same beds
3.	Cameron	a) Septic Tank b) Coke beds	Continuous Intermittent	Strictly anaerobic Chiefly aerobic
4.	Scott Moncrieff	a) Anaerobic Tank b) Coke tray filters	Continuous Continuous	Strictly anaerobic Strictly aerobic

It may be here remarked that it has been found impossible in practice to treat raw sewage by either of the first two processes. Under the forced aeration system it is necessary previously to remove the suspended solids as far as possible by chemical precipitation, or the filters will clog almost immediately.

Dibdin at first used a sewage which had been chemically treated, and found he could effect fair purification at the rate of a million gallons per day per acre. Later he simply removed the grosser particles by screening, but the solids still remaining in suspension reduced the efficiency of the beds by about one-third, so that not more than six or seven hundred thousand gallons per day per acre could be handled.

This production of sludge or, at the best, large amount of putrescible organic solid matter is naturally a serious disadvantage, but is overcome to a great extent by the methods of Cameron and Scott Moncrieff, where, on account of the more perfect hydrolysing action, the raw sewage can be used without any preliminary chemical treatment or screening. Not only are solid matters disposed of in the anærobic tanks, but in the effluent the soluble organic matter is in a more advanced condition than in the original sewage, so that a septic tank effluent is more readily purified in the coke beds than one from precipitated or screened sewage.

It has hitherto been said in a general way that anærobic action is necessary for the satisfactory liquefaction of solid organic matter, but a distinction must be drawn in this respect between that of animal and of vegetable origin. The former can be liquefied and disposed of by bacteria acting aerobically, though not so quickly as by anærobic action, but the latter is much more refractory. The solid vegetable matter, in domestic sewage at any rate, consists chiefly of paper and, to a lesser degree, of corks, rags, etc., whilst where there is much wooden pavement, and the combined system of drainage, as in London and many other English towns, there is in addition a large amount of woody fibre. The principal ingredient of all these is cellulose, and it is the cellulose which is refractory, requiring a considerable time for liquefaction even under the most favorable anærobic conditions. It now becomes evident why it is so necessary to screen the grosser particles, consisting chiefly of paper, corks, etc., before running the sewage into the Dibdin beds; why, even when this is done it is found in practice that the surface must be often raked over to break up the fibrous deposit which forms there, and why there is progressive loss of capacity, for there is no anærobic action possible on the surface of these beds, so that the cellulose caught there remains practically unchanged almost indefinitely.

A septic tank, however, overcomes the difficulty. There is always a deposit at the bottom, and a thick scum several inches thick on the surface. The scum prevents absorption of oxygen by the liquid, so that the action immediately underneath it is strictly anærobic. Paper, corks, and other solids, no matter how large, all go into the septic tank, where they either fall to the bottom, or rise to the top and adhere to the scum, remaining for days, weeks or months, until finally liquefied. The effluent is drawn from half-way down the tank (see diagram), so that no solids can escape with it, or at any rate only particles so small that even under subsequent slow working aerobic conditions they can be disposed of. When a septic tank is first started there is gradual formation of a deposit below and a scum above, and for a time these are constantly increasing in thickness, causing some loss of capacity of the tank. In 3 or 4 weeks, however, as the bacteria

multiply, an equilibrium is established, liquefaction balancing addition of solids, and after this is reached any additional loss of capacity is due to deposit, partly of mineral matter, which, of course, bacteria are powerless to remove, and partly of the already mentioned stable humus. The life of a septic tank may be put at two years, at the end of which time it must be cleaned out and started afresh. A Scott Moncrieff anærobic tank is filled with broken flints or other coarse material resting on a false bottom, whilst a Cameron septic tank has nothing of the kind in it. The reason for this difference now becomes obvious. In the upward filtration of the former, a deposit is formed in the lower chamber beneath the false bottom, and the deposited material is gradually cleared off as in the septic tank, but the lighter particles rise and rest against the under surface of the false bottom, where they are acted upon by the anærobics, and as soon as they get small enough, will pass through the meshes of the false bottom. Such meshes, however, to avoid clogging, must of necessity be somewhat coarse, so will allow comparatively large particles to pass. These must, therefore, be arrested in their upward course and the anærobics given a chance to work on them further before they are passed on to the beds. In the septic tank the lighter particles adhere to the scum which plays the part of the broken flints, so that these can be dispensed with. It has, however, been suggested that coarse material placed in the septic tank might accelerate bacterial action, as affording more surface for the bacteria to cling to, but any advantage gained in this way does not compensate for the loss of capacity, and the suggestion has not been carried out in practice. A priori then one would expect the septic tank to be more practical than the upward filtration, and that this is so seems to be proved by the more general adoption of the former; Scott Moncrieff tanks having been only exceptionally installed in England, whilst septic tanks are to be found all over the country.

The Dibdin system is now in use in many places in England, but it is found that a single contact bed is not sufficient to provide an effluent which will stand the incubator test. Double contact beds,

therefore, have to be provided, and at Hampton on Thames even triple contact beds (Diagram 1) are in use. Here, however, a specially good effluent is required, since one of the London Water Companies has its intake just below Hampton.

The method of working the contact beds, whether used with or without previous anærobic treatment, may be briefly described. The bed is filled slowly during one hour, the sewage allowed to remain "in contact" for 2 hours, then slowly drained off for one hour, and the bed allowed to "rest empty" for four hours, a cycle of 8 hours, after which it is again filled.

When a bed is first started the purification is slight, but the bacteria soon multiply enormously, clinging in colonies to the surfaces of the coke, and in 2 or 3 weeks the bed becomes ripe, after which it maintains its efficiency indefinitely if properly handled.

The effluent, however, is frequently submitted to the incubator test, and if at any time it shows signs of deterioration, the bed is given a rest for a week or two. In resting, the prolonged contact with the air weakens the anærobics, whilst the more strictly ærobic bacteria, which give the finishing touches to the purification, are strengthened, so that on resuming work the effluent improves again.

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Bacteria in the Soil.

Toward the close of the course in Bacteriology at Cornell Medical College, the subject of the soil bacteria is taken up, the students being given some garden earth to plate out, count the colonies, and estimate the number per gram. They are also told something about the Pathogenics found in the soil, but it seemed that, in addition to this, it would be of interest to them to get an idea of the various bacterial processes going on in the soil whereby the cycle of life is maintained, and to this end the following plan was evolved.

In the course of the previous exercises the students are shown how to test for nitrites with sulfanilic acid and naphthylamine (Griess-Ilovsay solution), and for ammonia with Nessler's solution, in order to determine if the bacteria they are studying are denitrifiers or not. The cultures are grown in the following solution:

Peptone,	.5 grams.
KNO_3,	2 grams.
NaCl,	2.5 grams.
Tap water,	1000 c. c.

After 6 or 7 days in the incubator the cultures are boiled for safety, 3 c. c. measured into an empty test-tube, and to this 2 c. c. of Griess-Ilovsay solution added. A red color shows the reduction of nitrates to nitrites. To the remainder of the culture a drop of Nessler's solution is added; a yellow color showing the presence of ammonia to which the nitrites may have still further been reduced.

Control tubes of the original solutions are, of course, also tested. The students then understand beforehand the tests for nitrites and ammonia.

To demonstrate the process of nitrification in the soil, the following solution is made up 6 or 8 weeks previously:

Microcosmic salt,	2 grams.
Potassium chloride,	1 gram.
Sodium chloride,	1 gram.
Magnesium sulfate,	.5 grams.
Water distilled,	1000 c. c.

This is distributed into several Erlenmeyer flasks, about 50 c. c. in each, and sterilized. Into some of the flasks a few grams of garden earth are introduced, and kept in the dark at room temperature. The inoculated flasks are tested at intervals for nitrites, and when these are found to be present in considerable quantities—the maximum is usually reached in 2 or 3 weeks—one-half the flasks are boiled, in order to stop further action, and can then be kept indefinitely, for demonstration of nitrosification.

In the remaining flasks the nitrites gradually become oxidized to nitrates by the nitrifiers, and in 6 or 8 weeks will give no greater reaction for nitrites than the controls, and may be tested for nitrates. For this, evaporate a little of the solution to dryness in a watch glass, add a few drops of phenol-sulfonic acid, and then a little 10 per cent. NaOH solution. A yellow color indicates the presence of nitrates.

We select them for our class: 1. A control flask. 2. One to show oxidation of ammonia to nitrites. 3. One to show oxidation of nitrites to nitrates.

By way of preliminaries to the demonstration of fixation of nitrogen some alfalfa plants are started growing 2 or 3 months beforehand, and the proceedings are as follows:

River sand is thoroughly washed, dried, and sterilized at 150° C. for two hours. Then put into garden pots (we used tin cases in which photographic platinotype paper had been stored), and boiled for an hour or so in the steam sterilizer. The alfalfa seeds are sterilized in 5 per cent. formalin for 15 minutes, washed in two or three changes of sterilized water, sown in the sand, and kept watered with sterile mineral solutions, containing no nitrogen, the one used by preference being:

Tricalcic phosphate, - - - - -	.5 gram.
Calcium sulfate, - - - - - -	.5 gram.
Sodium chloride, - - - - - -	.5 gram.
Potassium sulfate, - - - - -	.5 gram.
Magnesium sulfate, - - - - -	.5 gram.
Iron sulfate, - - - - - - -	Trace.
Water, - - - - - - - -	1000 c. c.

One-half of the seeded pots are supplied with pure cultures of nitrogen fixers.

FIG. 1.—Left.—Alfalfa Grown in Sterile Sand. Right.—Same with Cultures of Nitrogen Fixers.

isolated from alfalfa rootlets, two or three times during the first two or three weeks of growth, the remaining pots not being treated.

No precautions need be taken to prevent air bacteria from falling in during the period of growth, but it is advisable to keep the untreated pots in a separate room where no cultures of nitrogen fixers are handled. Conditions, however, of lighting and temperature should be the same for both.

The photographs (Figs. 1 and 2) show the appearance three months after sowing. It is true the growth is not very luxuriant in the treated pot, but a

laboratory window in the winter is not a favorable spot for plant life; and the difference between the two pots is sufficiently well marked for demonstration.

It is easy to obtain pure cultures of the nitrogen fixtures. The nodules are cut out from the rootlets, sterilized in the same way as explained for the seeds, pulverized, and the fragments plated out in gelatine. In 4 or 5 days the round non-liquefying colonies appear and can be transplanted. The bacilli will grow on ordinary media, but a specially favorable one is that recommended by Mazé. (Ann. Pasteur, 1897, '98, '99.)

Allow 100 grams dry weight of beans to germinate on moist cotton for two or three days, make an infusion of them in 1000 c. c water at 100° C. for 30 minutes; strain through cloth, add ½ per cent. NaCl, and neutralize. Boil one hour, filter, and add 3 per cent. of saccharose. The liquid comes out fairly clear and can be used as broth or can be made up with 10 per cent. gelatine.

On bean gelatine slants the bacteria grow in great profusion, and according

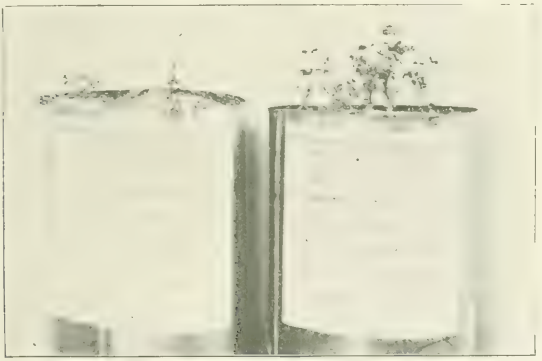

FIG. 2.—Left.—Alfalfa Grown in Sterile Sand. Right.—Same with Cultures of Nitrogen Fixers.

to Mazé they are able to fix nitrogen under these conditions, but this has not been tested by us for confirmation. Most investigators have been unable to get the bacteria to fix nitrogen in artificial cultures.

In the lecture attention is drawn to the fact that there is a constant accession of dead animal and vegetable matter to the soil, and this is disposed of by bacteria: the final products of the carbo-hydrates being gases and water, and of the nitrogenous substances, gases, water, and nitrates, the latter remaining in the soil.

In the course of explanation the following table is built up on the board:

	Changed to	By	By process of
1.—Insoluble proteids	Soluble organic substances	Anærobics	Hydrolysis
2.—Soluble organic	Gases, water and ammonia	Anærobics or Facultatives	Deoxidation (Putrefaction) Deoxidation or oxidation (Decomposition)
3.—Ammonia	Nitrites	Nitrosifiers	Oxidation
4.—Nitrites	Nitrates	Nitrifiers	Oxidation

At this point nitrification is demonstrated by means of the flasks prepared beforehand. Small portions of all three flasks are tested in tubes for ammonia and nitrites, and in watch glasses for nitrates: the differences between them being pointed out, and explanations given as to how the flasks were treated. The photograph (Fig. 3) shows: 1. Control. 2. Nitrosification. 3. The nitrites all oxidized to nitrates.

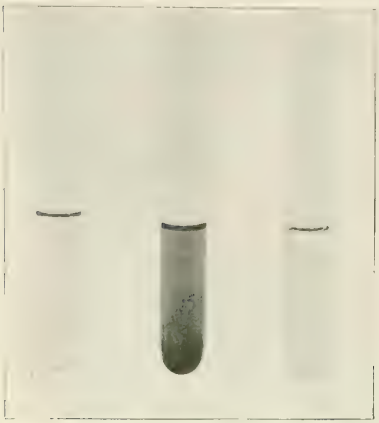

FIG. 3.—Center.—Nitrite Reaction with Griess-Ilovsay Solution. Left and Right.—No Reaction.

"Now green plants, speaking generally, can only utilize nitrogen when it is in the form of nitrates, so in this way the cycle of life is completed.

But on the way round there is considerable loss of nitrogen; much passes off as free N during the reduction of soluble matter to ammonia, and the same occurs to a great extent by the action of the denitrifiers. All the nitrogen therefore does not come back to the green plants in the form of nitrates and this loss must be made up somehow. Plants, animals, and bacteria we have so far considered can use and waste nitrogen, but they cannot fix it from the air. Nor is there in nature any fixation of nitrogen by chemical processes—or at least only to a very limited extent.

There is, however, a class of bacteria which can fix free N from the air under special circumstances, and hand on their nitrogenous surplus products to be utilized by certain green plants, —the leguminosæ, among which are peas, beans, clover, locust trees, etc. These bacteria are called nitrogen fixers and are universally present in the soil.

The seed of a leguminous plant when sown contains enough nitrogen to start the young plant growing. The stem shoots up a little way and the rootlets penetrate the soil to a consid-

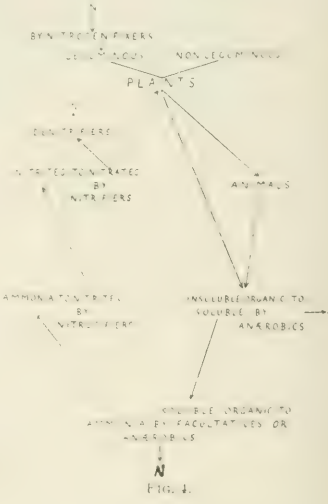

FIG. 4.

1979 erable depth before the young plant feels the necessity for more nitrogen. As the rootlets extend, the nitrogen fixers penetrate into them, and, growing there, form small nodules, which gradually increase in size as the bacteria multiply. See photograph (Fig. 5) of nodules on roots of locust tree.

FIG. 5.—Nodules on Roots of Locust Tree.

At first the bacteria grow at the expense of the plant, but when once they have made a good start they become able to make use of the free N of the air, forming certain nitrogenous products. Whether these products are in the form of nitrates or some other compound, is not yet determined, but at any rate the plant can utilize them, obtaining in this way nitrogen necessary for its growth.

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The plant continues to supply the bacteria with carbohydrates, which it can build up from CO_2 and H_2O, and in return receives the surplus nitrogen of the bacteria.

Leguminous plants, when they die, always return more fixed nitrogen to the soil than they take away from it, and in this way the cycle of life is perfected."

The plants are then shown to the class and cultures of nitrogen fixers are provided for examination.

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THE MICROSCOPIC STRUCTURE OF CORTICAL AREAS IN MAN AND SOME MAMMALS.

BY

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WITH 4 PLATES.

INTRODUCTORY.

The first step toward dividing the cerebral cortex into structural areas corresponding with the functional localization, determined by the experiments of Fritsch and Hitzig, was made when the giant cells of Betz were discovered and found to be characteristic of the motor area. These cells, described by various authors for man, were later identified by Bevan Lewis in the pig, sheep, cat and other animals, and found to be also present in isolated groups behind the motor area.

Nissl's method of staining nerve cells has, as I believe, furnished us with a means of carrying such investigation further than was possible by the older technique.

We are as yet unable to distinguish specific differences between cortical nerve cells. It is, for instance, impossible to show such a difference between cortical nerve cells in the auditory and those in the visual path. We can, however, say that the entire cortical visual center, considered as an organ, differs from the auditory or other cortical centers.

In order for us to determine whether any relation can be shown between the variations in the structure of the different regions in the cortex, on the one hand, and the function of these respective regions on the other hand, we must examine the largest possible number of brains, not only of those of one species of animals, but also those of animals in different stages of phylogenetic development.

From such comparative studies it has been found to be true that in animals whose organs of sense are equally well developed, but have not, on the whole, reached a high degree of specialization, no great differences in the structure of the cortex can be observed. This seems to be the case with the brain of the pteropus, the cat, and somewhat less so of the dog. On the other hand, in correlation with the special develop-

ment of a given sense organ, the corresponding cortical area presents a marked specialization as compared with the surrounding regions. In the brain of the horse, for instance, the olfactory region seems to be very highly developed, and is plainly distinguishable from the remaining cortex. The other organs of sense in that animal do not seem to have reached such a high degree of development, and the cortical areas corresponding to these organs are accordingly less developed and less marked. In the brain of the monkey and of man, we find several regions characteristically developed and sharply defined, a fact which corresponds to the development of the sense organs associated with these several regions.

Much confusion has arisen in comparing the findings of different investigators who have used dissimilar methods.

Another source of error lies, I believe, in the fact that only small pieces of the cortex taken from different regions were examined. There have never been made, to my knowledge, entire sections through the cerebral cortex, especially that of the human brain, for this purpose, and on that account a continued picture was not obtained. The sections which I have prepared were made through the entire hemisphere, frontally, horizontally, as well as sagittally, thus presenting a nearly continuous picture. A disadvantage of these entire sections, however, is met in the fact that in many places the cortex was not cut perpendicularly, and hence the cells assumed a different shape. These pictures can be easily recognized, however, if one has a little experience in microscopy of the cerebral cortex. The principal cause of the general lack of agreement as to the structure of the cortex is, however, due to the laying down of general conclusions drawn from a study of the brains of lower animals without sufficient comparison with the human cortex which is very specially constituted.

The investigations of Fritsch and Hitzig in 1870 followed by those of Munk, Ferrier, Hitzig, Goltz, Flechsig, Duret, Carville, Luciani, Tamburini, Horsley, Schäfer and others have furnished us with a solid experimental basis for a localization of function in the cerebral cortex of the lower animal; while the results of human clinico-pathological cases, which have lately been examined as extensively as our methods will permit, have made it possible to institute valuable comparisons here. If, however, we undertake to compare the results of our experimental investigation of animal brains with those obtained in clinico-pathological cases of human brains, we must not forget that the different centers vary in animals and man and also among animals themselves, with regard to their situation in the cortex. Such variation is to be explained by the fact that a specially developed cortical center in

an animal will probably occupy more space than those not so highly developed, and thus displace the other less developed centers, so that these may come to occupy a position different from that in other animals. Thus according to the experimental investigations of Munk, the visual center in the monkey of lower types is in the operculum; but an attempt to locate it in man in the corresponding region would result in great confusion, as it is not situated on the lateral surface of the hemisphere in man as in the monkey, but on the medial surface. This displacement of the visual center in man is very likely brought about by the more highly developed association centers which occupy the lateral surface of the hemisphere in the parietal and occipital lobes.

It is true, some investigators still adhere to the idea that the visual center is situated on the lateral surface of the hemispheres, but such is not the case in man.

As a result of one or more of the sources of error just enumerated we find the following greatly differing views maintained:

Meynert found five layers almost everywhere in the cortex, except in the occipital lobe where he described eight layers. Other investigators, as for instance *Ramón y Cajal*, consider only four layers as the common type, the cortex presenting, however, here and there a few slight variations which can yet be considered part of the four-layer type. In his later writings, *Ramón y Cajal* differs slightly from his former opinion. He says, concerning the sight-center in the occipital lobe, "The investigations which I have made on the human cortex, as well as on that of the dog and cat by both Nissl and Golgi methods, have led me to distinguish the following layers:

1. Plexiform layer (called molecular layer by authors generally, and "cell-poor layer" by Meynert).
2. Layer of small pyramids.
3. Layer of medium-sized pyramids.
4. Layer of large stellate cells.
5. Layer of small stellate cells. (Called "layer of granules" by other authors.)
6. Second plexiform layer, or layer of small pyramidal cells, with arched axon.
7. Layer of giant pyramidal cells (solitary cells of Meynert).
8. Layer of medium-sized pyramidal cells, with arched ascending axon.
9. Layer of fusiform and triangular cells (fusiform-cell layer of Meynert).

In the human motor cortex *Ramón y Cajal* recognizes the following layers:

1. Plexiform layer (layer poor in cells of Meynert, molecular layer of some writers).
2. Layer of small and medium sized pyramids.
3. External layer of giant pyramids.
4. Layer of small stellate cells. (Granule-cell layer of authors.)

5. Internal or deep layer of giant pyramids.

6. Layer of polymorphic cells (fusiform and medium-sized pyramids of some writers).

Von Monakow seems to share the view formerly expressed by *Ramon y Cajal*. *Golgi* describes three layers, and even the cortex of the occipital lobe, according to this author, contains only three layers.

Koelliker divides the cortex into four and six-layer types, as follows:

1. The white cortical layer of the stratum zonale, poor in cells.
2. Layer of small pyramidal cells.
3. Layer of medium-sized and large pyramidal cells.
4. Layer of polymorphous cells.

In many convolutions, he found, as did Hammaberg, six layers, thus:

1. Stratum zonale.
2. First layer of small pyramidal cells.
3. First layer of medium-sized and large pyramidal cells.
4. Second layer of small pyramidal cells.
5. Second layer of medium-sized and large pyramidal cells.
6. Layer of polymorphous cells.

Obersteiner assumes a type of five layers:

1. Layer of neuroglia cells, or stratum moleculare.
2. Layer of small pyramidal cells of outer nerve-cell layer.
3. Layer of large pyramids, formation of the corun ammonis or middle nerve cell layer.
4. Layer of the small irregular cells, granule formation, or mixed nerve-cell layer.
5. Layer of spindle cells, claustral formation.

According to *Obersteiner*, a few slight variations occur which are, however, not so important as to prevent their inclusion in the five-layer structure. He is unable to find the eight layers of Meynert, and believes that nowhere, and especially in higher animals, do sharp differences appear in the structure of the cortex.

Bevan Lewis described, in the cortex, a structure of five and six layers. The five-layer structure is found in man and monkeys in front of the central sulcus, corresponding to the anterior central gyrus. In the lower animals which he examined, he found it surrounding the fissura cruciata. This five-layer type is, according to him, characteristic of the motor area. The six-layer type is found behind the central sulcus and is believed to be characteristic of the sensory cortex.

Hammaberg, who made a great number of accurate investigations of the human cerebral cortex, seems to incline towards the opinion of Lewis.

COMPARATIVE ANATOMY OF CORTICAL REGIONS.

I shall now endeavor to describe the cortex of various animals, comparing the functional centers according to structure and localization. I have examined sections of six different animals, which are phylogenetically fairly well separated from one another.

1. Pteropus. 2. Horse. 3. Cat. 4. Dog. 5. Monkey (Macacus Cynomolgus), and 6. Man.[1]

Olfactory Region.

The olfactory center is of variable extent in the animals examined, being largest in the horse and smallest in man. That portion of the cortex which can undoubtedly be considered the olfactory cortex, presents in the pteropus, horse, cat, dog, monkey, and man, a rather characteristic structure, which is due to the fact that the cells in the outer polymorphous cell layer, arrange themselves in smaller and larger groups, thus giving to this layer an interrupted appearance. In some places, especially in the olfactory tract, these cells lie so close together (not in groups) and are so deeply stained, that one can distinguish plainly between this layer and the same layer in other cortical centers.

In the pteropus, this peculiar structure extends over a comparatively large area of the ventral and lateral parts of the hemisphere; its limits are difficult to describe precisely, owing to the fact that this brain contains no fissures which could be used as landmarks.

In the dog, cat, and monkey, this type is limited to the hippocampal gyrus and seems to extend into the posterior portion of the gyrus fornicatus; it then passes into the olfactory tract, where nearly all the pyramidal cells disappear, only a few polymorphous cells remaining in place of the pyramidal cells.

In the human cortex this type is confined to a very small area in the hippocampal gyrus bordering on one side, on the formatio cornu ammonis, of which it seems to be the continuation, while on the other side it is in contact with the seven-layer type.

One can plainly see, when approaching the olfactory region from the seven-layer type (to be described under a separate heading), the granule-cell layer disappear and the pyramidal cells diminish in number and finally seem to be transformed into polymorphous and spindle shaped cells, which lie between the characteristic outer layer and inner layer of polymorphous cells.

In the cortex of the horse's brain, this type occupies a more promi-

[1] Of the horse and of man, I lost considerable material, especially the temporal lobe of man, so that at present my sections will not suffice to give a complete picture of the cortex of horse and man. I have, however, noted in the body of the text certain observations, and should add here that, in a horse's brain, the finer structure differs from that of the cortex of other animals in that one sees clearly, with the exception of a few limited regions, six layers in which two stripes, the stripes Gannari or Baillarger, can be plainly seen.

nent position; it is present in the gyrus fornicatus where the latter passes into the hippocampal gyrus, and in the markedly developed anterior portion which passes from the medial to the lateral frontal surface of the hemisphere. This addition to the gyrus fornicatus gives to the brain of the horse the aspect of possessing a large frontal lobe. Such, however, is not the case, for in the brain of the horse the characteristic structure of the frontal lobe is absent. I believe we may consider this extensive cortical region as a part of the olfactory center. A peculiar feature of this region, however, is the presence of large motor pyramidal cells, evidently signifying that the olfactory and motor centers are here merging into one another.

Motor Area.

This area is mainly distinguished by the large pyramidal cells of Betz. These giant cells are not similar in shape or in size in different animals. In the horse they average about 0.44 mm. in length and 0.374 mm. in width. In the pteropus the length of the largest cell is 0.36 mm. and its width at the base 0.18 mm. In the cat, however, these giant cells measure 0.612 mm. in length and about 0.468 mm. in width at the base of the cell. In the dog they measure 0.72 mm. by 0.324 mm.; in the monkey, 0.468 mm. by 0.316 mm.; in man, 0.54 mm. by 0.36 mm. These large pyramidal cells of the human cortex frequently show a mass of pigment surrounding the nucleus, which I have not seen in pyramidal cells of lower animals.

In the cortex of the pteropus, the cat, the dog, the monkey and of man, the motor area presents a structure of five layers.

In the horse we can also assume five layers, but the inner stripe of Baillarger is here so clearly defined that it can be considered a special layer, making a six layer type, though such a distinction would be arbitrarily based on the position of the large pyramidal cells in the upper portion of the stripe of Baillarger, leaving the lower portion poor in cells, and giving it the appearance of a distinct layer.

In the motor area of the human cortex, there is present a suggestion of the granule-cell layer. This granule-cell layer does not appear clearly if at all in the motor area of lower animals.

The motor cortex in the dog, the monkey and in man (less clearly so in the cat, horse and pteropus) is much wider than the sensory cortex lying behind it, but the cells in the sensory cortex lie much more closely together and are also much smaller.

The ratio of the depth of the motor cortex compared with the sensory cortex is in:

Dog as	2.210 : 1.690
Monkey	2.340 : 1.590
Man	3.510 : 1.820

It is evident from these figures that this difference between the motor and sensory cortex is least in the dog and most in man.

In the pteropus, the large cells are distributed over a relatively large cortical area in the anterior portion of the hemisphere on its lateral as well as on its medial surface. They are situated in the fourth layer of the five layer type. For the reason that relatively large cells are found in the pteropus in such large numbers, and are spread over so large an area, I am inclined to doubt that these really represent the motor cells only. These cells, being in a parapyknomorphic state, do not differ from the other pyramidal cells in the same region, whereas the motor or large pyramidal cells in higher animals are in a pyknomorphic state, compared with the parapyknomorphic state of the smaller pyramidal cells in the same region.

In the cat and the dog, these cells lie in a limited area surrounding the fissura cruciata.

In the monkey, they lie in the well defined anterior central gyrus, but isolated cells are still found in the posterior central gyrus. They do not, however, reach as far ventralward in the posterior central gyrus as the stem of the fissure of Sylvius, but only as far as the lower extremity of the central sulcus of Rolando, which is shorter comparatively in the monkey than in man. The large pyramidal cells in the posterior central gyrus are not so large as the same cells in the anterior central.

In the human cortex, the large pyramidal cells are found in the anterior central gyrus. Both in size and in numbers they reach their highest point of development in the dorsal portion of the anterior central, but diminish in both respects as they approach the ventral extremity of this gyrus. Anteriorly from the anterior central gyrus they occur only in small numbers in the posterior extremity of the upper and middle frontal gyri.

On examination of the motor area, with a low power lens (a. a. Zeiss), one observes the peculiar fact that in lower animals the large pyramidal cells are crowded closely together and presumably on that account occupy a limited area, while in man the cells are more scattered and cover a comparatively larger area of the cortex. Posteriorly from the anterior central gyrus, the boundary being formed by the central sulcus (fissure of Rolando), the motor type passes abruptly into the seven layer type, while toward the frontal lobe the transition into the seven layer type is gradual. This latter type in the frontal lobe resembles the parietal

type in that it can be divided into seven layers, but differs from it with regard to the structure of the pyknomorphous or deeply stained pyramidal cells.

The motor cortex of the animals studied presents the following five layer type:

1. Layer of glia or tangential fibers.
2. Outer layer of polymorphous cells.
3. Layer of parapyknomorphous pyramidal cells.
4. Layer of large pyknomorphous pyramidal cells (Betz's pyramidal cells or Lewis' ganglionic cells).
5. Inner layer of polymorphous cells.

The pyramidal cells of the third layer are distinguished not only by their smaller size, but also by the structure of their chromatic substance, which is more or less reticular. We can therefore designate these cells according to Nissl's nomenclature as arkyochrome cells. The large pyramidal cells of the motor area, on the other hand, as well as a great number of the pyknomorphous cells of the fourth and sixth layers of the parietal and temporal regions present the characteristic stichochrome structure.

The Seven-Layer Type.

It is difficult to give this type a functional name, because it extends, in the human brain, over a very large area, in which we must look for more than one function. In the human brain it is found in—

(1) The whole parietal lobe, which we can assume with tolerable certainty to be in part the cortical center of sensation.

(2) The entire lateral portion of the occipital lobe.

(3) The whole frontal lobe.

(4) The temporal lobe, wherein a few modifications are found.

(5) The præcuneus.

(6) The region of the island of Reil, where again some slight modifications occur.

It is probable that in this cortex are situated the different centers of association, which have been described by Flechsig.

In the brain of the monkey this type is comparatively less extensive in proportion to the well defined motor and visual centers.

In the cortex of the dog and cat, it occupies a much smaller area and is not so clearly defined. Owing to the absence of the frontal lobe in these animals, this type is entirely wanting in front of the motor zone.

In the Pteropus this type is nowhere present in the cortex.

The seven layer type may now be compared with the five layer type (motor area) as follows:

The first layer (layer of glia and tangential fibers, molecular layer) is similar in structure to that of the motor area, but is narrower. The second layer is similar to that in the motor area. The third is also similar to that of the motor region, but is much narrower in many places, especially on the convexities of the convolutions. The fourth is a layer of pyknomorphous pyramidal cells, which can be distinguished from the third layer by the more intense staining of the cells. This layer does not exist as such in the motor region. The fifth layer is Hammaberg's fourth layer, or the layer of granule cells of other authors. Koelliker named it the second layer of small pyramids. This layer does not exist in the motor region of the brains of the lower animals I have examined; in man just a trace of it is seen in the motor region, but it is not sufficiently developed to permit one to distinguish it as a separate layer. The sixth layer is again a layer of pyknomorphous pyramidal cells, the ganglionic cells of Lewis. This layer seems to be a continuation of the fourth layer of the motor region. The seventh layer is the inner layer of polymorphous cells. This layer corresponds to the fifth of the motor region.

We therefore have the following layers in the seven layer type:

1. Layer of glia or outer layer of tangential fibers.
2. Outer layer of polymorphous cells.
3. Layer of parapyknomorphous pyramidal cells.
4. Outer layer of pyknomorphous pyramidal cells.
5. Layer of granule cells.
6. Inner layer of pyknomorphous pyramidal cells.
7. Inner layer of polymorphous cells.

In the dog, the monkey and in man, the transition from the motor to the seven-layer type can be distinctly recognized. In man, monkey, and dog, less distinctly in the cat, the large pyramidal cells of the motor area become smaller at the point of transition and arrange themselves in two rows, between which the granule-cell layer (fourth layer of Hammaberg, Ramón y Cajal's layer of small stellate cells) is distinctly seen, giving to this type its characteristic appearance.

The granule-cell layer is always present in this type. The two layers of pyknomorphous pyramidal cells, however, are subject to variation. There are places in the cortex where the outer layer of pyknomorphous pyramidal cells is wanting and their place is taken by the parapyknomorphous pyramidal cells of the third layer, so that in those places the fourth layer is absent. Then again, there are places where the inner pyknomorphous pyramidal cells are missing and where, more or less clearly, can be seen a light stripe, which is known as the inner stripe of Baillarger. This condition is most frequently found in the human

cortex, so that it can be accepted as the rule there. In the dog and monkey, on the other hand, the inner layer of pyknomorphous pyramidal cells is very distinctly developed and spread over a fairly large area. Now if, as is generally done, small pieces of the cortex are cut here and there from the surface of the hemispheres, it may happen that either the outer or the inner layer of pyknomorphous pyramidal cells alone is present (in most places the outer layer, as it is present almost everywhere in the seven-layer type), a study of such pieces might lead one to believe that the inner or outer layer of pyknomorphous pyramidal cells does not exist at all. In those places where the internal layer of pyknomorphous pyramidal cells is absent, it is difficult to recognize a seven-layer type, if by absence of these cells the stripe of Baillarger is not plainly brought to view. I have not as yet been able to definitely fix the exact points, where these variations occur, but one can see that these pyknomorphous pyramidal cells are present in greater numbers in the dorsal than in the ventral portion of the parietal lobe. It is also noticeable, that the inner layer of pyknomorphous pyramidal cells disappears as the occipital lobe is approached, so that posteriorly the stripe of Baillarger comes more into view.

The seven-layer type is found in the dog, behind the fissura cruciata, and extends backward to within 2 cm. of the occipital pole. It is found less sharply marked in the temporal lobe and is entirely absent in front of the fissura cruciata.

In the monkey the seven-layer type is most distinctly marked in the parietal region lying between the central sulcus and the external occipital fissure, or ape cleft. In the temporal lobe this type undergoes a modification, which will be described below. In front of the fissure which corresponds to one of the frontal fissures in man (Munk calling it "Harkenfurche" in the monkey), this type is again evident and its limits coincide with those of the frontal lobe.

In the human cortex, this type extends, as I have stated before, over a very large area. It is found in the parietal lobe and in the præcuneus. It occupies the entire lateral surface of the occipital lobe, and extends, modified, into the temporal lobe.

On examining this type, the question suggests itself, what may the function of this seven-layered cortex be? Extirpation experiments performed on the Monkey, especially those of Munk, seem to show that the muscular sense is located in the parietal lobe. But he points out two areas in the monkey showing this type, the destruction of which is not followed by any focal symptoms. Of these two cortical regions, one lies in front of the frontal sulcus, Harkenfurche, which undoubtedly corresponds to the frontal lobe in man, and the other is a small area at the posterior dorsal extremity

of the inter-parietal sulcus in front of the ape cleft. The observations in regard to this type in the human cortex do not agree and are not conclusive as Von Monakow points out. His discussion of the three cases of Vetter, Grasset, and one of his own, together with a later case of Starr and McCosh, permit us to conclude that it is fairly probable that in the monkey and man, the parietal area is in part the center for muscular sense, and as the sensory fibers (continuation of the lemniscus) run into the parietal lobe, the center for tactile, pain and temperature sense lies in part also there and probably extends to the anterior central gyrus.

In the frontal lobe, lesions have occurred accompanied by no focal symptoms, but in larger lesions confined to this region, peculiar changes in the individual's character have been observed, such as loss of the sense of morality and decency.

It has been found that all these areas show a nearly similar structure, namely, that of seven layers. In how far are we able to say they resemble each other functionally? Flechsig, basing his opinion upon his investigations of the development of the medullary sheaths, declared the parietal lobe to be one of four association centers; the other three being in the frontal lobe, in the region of the island of Reil, and in the temporal lobe Flechsig also found, that the structure was the same in these different regions.

That the parietal lobe is only an association center, as is believed by Flechsig, seems to be incorrect, considering the clinico-pathological observations, and, according to the investigations of v. Monakow, the fibers continue from the lemniscus, extend principally into the parietal lobe. Nevertheless, the structure of the parietal cortex is similar to that of the frontal cortex.

The investigations of Golgi, Martinotti, Ramón y Cajal and Koelliker have shown, that in the layer of granule cells are found the cells of Martinotti, or that most of these granule cells are Golgi cells of type II. The Martinotti cells possess ascending axones, which are partly distributed in the outer layer of tangential fibers, and partly end in horizontal fibers before reaching the outer layer. We can, therefore, judging from the course of the axones, say that these cells do not send out centrifugal impulses.

The granule cells present the smallest numerical development in the motor area, but are found in great numbers in the parietal region, where they form a new layer. These cells are most numerous in the visual center, which undoubtedly is the most specialized center in the cortex of man and monkey.

These granule cells—judging from their anatomical structure, from their position in the cortex, and from the fact that they are found in greater numbers in those centers in the cortex, in which probably a greater number of centripetal impulses are received, while they occur in less numbers in those centers in the cortex, in which probably fewer centripetal impulses are received—probably are intermediate cells, which receive the centripetal impulses, and transmit them further by their ascending axones to the dendrites of the pyramidal cells (association and projectional cells), whence they reach their destination in a descending direction. Such impulses need

not come from the periphery, but may come from different regions of the cortex.

Auditory Region.

This center is, judging from its structure, still a part of the seven-layer type, but as the localization is known with a fair degree of certainty, I will describe it, as others have done, as the auditory center.

Experimental investigation made on animals has shown that the temporal lobe is the seat of the auditory center. Thus, Munk has caused deafness by extirpating the temporal lobe in monkeys; and in man, on the basis of clinico-pathological observations, the auditory center was also located in the temporal lobe. Flechsig has followed the auditory fibers from the lower auditory centers directly to the transverse temporal gyri and to the superior temporal gyrus opposite the transverse gyri.

I have succeeded in preparing sections of the temporal lobe of the pteropus, cat, dog and monkey; but in the brain of the horse the stain of the sections was not a good one, and of the human brain the greater part of the temporal lobe was injured in preparing. I shall, therefore, be able to describe this, as well as several other portions of the cortex in man and the horse, only later on.

In the pteropus I have not been able to find any important differences from the general type; in the cat and dog indistinctly; but in the monkey I have found more plainly the following deviations from the contiguous parietal type. In the temporal lobe, principally two layers of cells increase in size—the granule layer and inner layer of polymorphous cells. One can also notice that in the inner layer of pyknomorphous pyramidal cells the pyramidal cells increase in number, this being not so conspicuous as the increase in the two aforementioned layers. The inner layer of pyknomorphous pyramidal cells adjoins a little more closely the granule-cell layer, and therefore the stripe of Baillarger is plainly visible between the inner layer of polymorphous cells and the inner layer of pyknomorphous pyramidal cells, thus suggesting a structure of eight layers. *The most striking feature of the cortex in the auditory region* is the enormous increase of cells in the granule cell layer, and in the inner layer of polymorphous cells, as compared with what is found elsewhere in the seven-layered cortex.

Visual Center.

The visual center has aroused the greatest interest on account of experimental and clinical observation. But up to the present, it still seems unsettled as to where it is located and how far it extends in the

cerebral cortex. The greatest number of experiments regarding the localization of this center in the cerebral cortex of animals have been made by Munk and Ferrier.

Ferrier considers himself justified in the belief that the visual center is situated in the angular gyrus.

According to *Munk* the visual center is found in the occipital lobe. In the dog it does not, however, extend as in the monkey to the occipital pole, but is believed by him to be situated more anteriorly and laterally.

Where is the visual center situated in the human cortex? Many investigators have endeavored to answer this question, but unfortunately the observations of these investigators have not in all cases led to the same results.

Henschen concludes that the visual center is confined exclusively to that region of the cortex which is situated around the anterior two-thirds of the calcarine fissure.

According to *Viallet* the visual center is found in the entire mesial surface of the occipital cortex, and does not extend over the convex surface of the hemisphere.

Seguin locates the visual center in the cuneus only.

Von Monakow believes that all facts necessarily speak in favor of the view that the visual area occupies besides the entire cortex of the occipital convolutions proper (cuneus, lingual lobe, gyrus descen. 01-03), also at least the posterior portion of the angular gyrus.

Other investigators, as *Ferrier, Gowers, Angelucci, Bianchi, Luciana,* and many others locate the visual center on the lateral as well as medial surfaces of the hemisphere.

It is evident, from all these differing opinions, that the visual center has not yet been located with certainty in the human cortex.

Structure.—The visual center in the cortex of the monkey has been determined beyond any doubt, and accurately located by the experiments of Munk. This sphere is situated in the occipital lobe behind the parieto-occipital fissure, or ape cleft. I have shown that anatomically this cortical sphere presents a typical and characteristic structure and can clearly be distinguished from adjoining areas. This sudden transition in structure from the parietal to the visual cortex is very striking and sharply marked. It is situated at the posterior margin of the ape cleft, in the depth of which the parietal type is still present.

A comparison of the cortex of the visual region with the adjoining cortex of the parietal region, reveals first of all the fact that the radiating fibers in the cortex do not appear in the visual region as is so plainly the case in the parietal region; and consequent upon that fact the first layer, or layer of tangential fibers, is not as wide here as in the parietal region. In addition, it may be noticed that everywhere in the visual

cortex an enormous number of granule cells appear. The second, third and fourth layers can at first sight be distinguished only indistinctly; but after a more careful examination of the field one does see that in the second layer the cells are set more closely together than in the third, and, owing to that again, the fourth layer becomes more conspicuous. Besides, there are in this fourth layer somewhat larger cells than in the second and third. Below the fourth layer one sees very plainly a layer of tangential fibers (the stripe of Baillarger), in which here and there are found large, fusiform, irregular cells. Then, below this layer, there is a very conspicuous and marked granule-cell layer. Then follows another layer of tangential fibers (inner stripe of Baillarger), in which again here and there are found large fusiform cells. Lastly, the inner layer, or layer of polymorphous cells, differs from the layer of polymorphous cells in the parietal and motor regions. It is almost twice as wide as in the above mentioned two types; is more sharply marked by the great number of cells found therein, and besides there are seen scattered here and there large, intensely stained, irregular cells which have been described by Meynert, who named them "solitary cells."

There are, therefore, six layers, if we consider the second, third and fourth layers as one. If we, however, count each one separately, then there are eight layers, as follows:

1. Layer of glia cells, or outer layer of tangential fibers.
2. Outer layer of polymorphous cells.
3. Layer of parapyknomorphous pyramidal cells (here, however, the cells have a more irregular shape).
4. Layer of granule cells, with somewhat large, irregular cells, among which are also found pyramidal cells.
5. Layer of tangential fibers (outer stripe of Baillarger), in which stripe irregular cells are found.
6. Layer of granule cells (inner layer).
7. Layer of tangential fibers (inner stripe of Baillarger).
8. Layer of polymorphous cells (inner layer).

In the dog and the cat the visual cortex is not plainly distinguishable from the remainder of the cortex. One can see, however, that it contains more granule cells than the parietal cortex. *Another fact which strikingly coincides with this is the corresponding lack of development of the lateral geniculate body in the brain of the dog and cat*

In the monkey, whose visual cortex is very highly developed, the development of the lateral geniculate body is correspondingly more marked than in the dog and cat. The lateral geniculate body of the human brain is like the monkey's.

It is very likely that the phylogenetic secondary sight centers (cortex) of the dog and cat have not relieved the phylogenetic primary sight centers (Thalamus and corpora quadrigemina) of their functions (in the sense of Steiner) to such a degree as in the brain of the monkey and man; hence the lack of development of the cortex and lateral geniculate bodies in dog and cat. It is possible that Goltz was not entirely wrong when he said that after the destruction of the entire cortex of the dog he could still see to some extent.

If this cortex, which presents such a characteristic structure in the monkey, and which is so sharply and so plainly differentiated from the adjoining parietal cortex, alone represents the visual cortex in the monkey, why should it not likewise alone represent the visual cortex in man? We may assume, with fair certainty, this to be the case. As the visual region can be sharply defined by its structure, its localization also ought to be definitely fixed. It has been demonstrated that the visual region nowhere passes over the margin of the longitudinal fissure onto the convex surface of the hemisphere, and that in the cases examined by myself, it assumes a pyramidal shape. The point of the pyramid lay in the anterior extremity of the calcarine fissure, the base posteriorly at the margin of the longitudinal fissure. The upper side of the pyramid projected as far as above the middle of the cuneus, the lower side down to the gyrus fusiformis and partly into it.

It is possible that the visual region varies in different brains. This variation may be due to the calcarine fissure, the course and extent of which is variable in different brains.

GENERAL.

In conclusion, a few general remarks on the arrangement of the cells of the cerebral cortex seem desirable.

In the human cortex, the seven-layer structure seems to coincide, according to its situation, with the association centers of Flechsig. He says:

"The plainly evident differences in the structure of the cortex of the central gyri, the calcarine fissure, the hippocampal gyrus, etc., have already been known for a long time, though strange to say they have not been sufficiently appreciated. On the other hand, it requires subtle research to demonstrate, for instance, differences between the cortex of the second frontal gyrus of the inferior parietal gyrus, of the second temporal gyrus, etc. If we measure the ganglion cells for their largest sizes, we obtain differences which can be expressed in figures, to be sure, but the type, as a rule, remains the same. We must, however, not overlook the fact that the border regions of sensory and association centers present occasionally

transitional types. The cortex of each sensory area is evidently composed of two differently constructed sets of layers:

1. The layers of specific elements (the spindle cells of the gyrus fornicatus, the granule cells of the visual sphere, the giant cells of the central gyri, the cylindrical cells of the auditory area, etc.); and

2. The elements of the association centers which spread over the entire cortex.

The association centers contain in their cortex elements only of the latter kind, but these layers also pass into sensory centers, so that the latter frequently present slight resemblances of the structure of the association centers.

I cannot agree to everything Flechsig says here. That in some places, as, for instance, in the motor area, giant cells and in the cornu ammonis characteristic cells are found, is, no doubt, plainly evident. But I cannot agree with him when he says, that in the visual area only, granule cells are found, or that in the auditory region only cylindrical cells are found, or that only in the anterior portion of the gyrus fornicatus are found the large characteristic spindle cells. The granule cells which he finds only in the visual region, are found almost everywhere in the cerebral cortex, not in equal numbers, however, as I have already pointed out. The cylindrical cells, which he claims to have found only in the auditory area, I have not been able to find in that part of the temporal lobe, of which I have obtained good specimens, and some of the sections have surely been made through the auditory area.

The main distinction between the different cortical regions does not appear so much in the individual cells as it does in the composition of the entire cortex. It is true that the large giant pyramidal cells are very characteristic of the motor area, and in the visual area those cells, which may be considered analogous to the pyramidal cells in the adjoining areas, are no more so distinctly pyramidal in shape. These modifications can probably be brought into accord with the following facts: I have already pointed out that in the visual area the cells are no more arranged in rows perpendicularly to the upper surface of the cortex, and that the fiber striations are no longer so conspicuous. We may conclude from this, that the radiating fibers are not developed so well in the direction of the outer layer of tangential fibers as in the stripes of Baillarger. In consequence, these two inner layers of tangential fibers, or stripes of Baillarger, are enormously developed. (The fifth and seventh layers of the eighth layer type.) This is followed by, or is the result of, the fact that the cells do not send one large dendrite to the periphery, giving them a pyramidal shape, but send many dendrites to the inner layers of the tangential fibers, for which reason they become more polymorphous.

One also sees in the innermost, or eighth layer, the solitary cells of Meynert, which were not found to be so large in any other place in the layer of polymorphous cells.

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DESCRIPTION OF FIGURES ON PLATES I, II, III AND IV.

Fig. 1. Olfactory cortex of *man*.

A. Layer of glia cells and outer layer of tangenital fibers.
B. Outer layer of polymorphous cells, cell group formation.
C. Mixed cells.
D. Inner layer of polymorphous cells.

Fig. 2. Olfactory cortex of the *Pteropus*.

B. The characteristic formation of cell groups in the outer layer of polymorphous cells.

Fig. 3. Olfactory cortex of the *dog's* brain.

B. The characteristic formation of cell groups in the outer layer of polymorphous cells.

Fig. 4. Cortex from the anterior portion of the gyrus cinguli (Limbic lobe), where the latter passes onto the convex surface of the hemisphere. (Presumably, the olfactory region in connection with the motor cortex.) *Horse's* brain.

A. Layer of glia cells and outer layer of tangential fibers.
B. Outer layer of polymorphous cells, showing the characteristic cell group formation.
C. Layer of parapyknomorphous pyramidal cells.
D. Layer of pyknomorphous pyramidal or motor cells.
D¹. Inner stripe of Baillarger.
E. Inner layer of polymorphous cells.

Fig. 5. Cortex from the motor area of the *dog's* brain.

1. A. Layer of glia cells and outer layer of tangential fibers.
2. B. Outer layer of polymorphous cells.
3. C. Layer of parapyknomorphous pyramidal cells.
4. D. Layer of large, or pyknomorphous pyramidal cells.
5. E. Inner layer of polymorphous cells.

Fig. 6. Cortex from the motor area of the brain of the *monkey*. (Anterior central gyrus.)

1. A. Layer of glia cells and outer layer of tangential fibers.
2. B. Outer layer of polymorphous cells.
3. C. Layer of parapyknomorphous pyramidal cells.
4. D. Layer of large, or pyknomorphous pyramidal cells.
5. E. Inner layer of polymorphous cells.

FIG. 7. Cortex from the motor area of the *human* brain.

1. A. Layer of glia cells and outer layer of tangential fibers.
2. B. Outer layer of polymorphous cells.
3. C. Layer of parapyknomorphous pyramidal cells.
4. D. Layer of large, or pyknomorphous pyramidal cells.
5. E. Inner layer of polymorphous cells.

FIG. 8. Cortex from the parietal lobe of the *human* brain.

1. A. Layer of glia cells and outer layer of tangential fibers.
2. B. Outer layer of polymorphous cells.
3. C. Layer of parapyknomorphous pyramidal cells.
4. D. Outer layer of pyknomorphous pyramidal cells.
5. E. Layer of granule cells.
6. F. Inner layer of pyknomorphous pyramidal cells.
7. G. Inner layer of polymorphous cells.

FIG. 9. Cortex from the parietal lobe of the *monkey*.

1. A. Layer of glia cells and outer layer of tangential fibers.
2. B. Outer layer of polymorphous cells.
3. C. Layer of parapyknomorphous pyramidal cells.
4. D. Outer layer of pyknomorphous pyramidal cells.
5. E. Layer of granule cells.
6. F. Inner layer of pyknomorphous pyramidal cells.
7. G. Inner layer of polymorphous cells.

FIG. 10. Cortex from the parietal lobe of the dog's brain.

1. A. Layer of glia cells and outer layer of tangential fibers.
2. B. Outer layer of polymorphous cells.
3. C. Layer of parapyknomorphous pyramidal cells.
4. D. Outer layer of pyknomorphous pyramidal cells.
5. E. Layer of granule cells. (Layer of small stellate cells, by Ramón y Cajal.)
6. F. Inner layer of pyknomorphous pyramidal cells.
7. G. Inner layer of polymorphous cells.

FIG. 11. Showing the transition from the seven-layer type to the visual area or eight-layer type in the *monkey*.

7. T.—Seven-layer type.
8. T.—Eight-layer type, or visual region.

The seven-layer type is as in Fig. 9.

Eight-Layer Type.

1. A. Layer of glia cells and outer layer of tangential fibers.
2. B. Outer layer of polymorphous cells.
3. C. Layer of parapyknomorphous pyramidal cells.
4. D. Layer of granule cells, with fusiform or irregular cells scattered here and there.
5. E. Inner layer of tangential fibers. (External stripe of Baillarger.)
6. F. Layer of granule cells.
7. G. Inner layer of tangential fibers. (Inner stripe of Baillarger.)
8. H. Inner layer of polymorphous cells.

Fig. 12. Showing the transition from the seven-layer type to the visual area, or eight-layer type in *man*.

7. T.—Seven-layer type.

8. T.—Eight-layer type, or visual region.

The layers are the same as in the monkey. Here one can see the solitary cells in the inner stripe of Baillarger.

Fig. 13. Frontal section through the lateral geniculate body of the *dog*, simple and slightly differentiated. The same in the cat.

Fig. 14. Frontal section through the lateral geniculate body of the *monkey*, very much more differentiated than that of Fig. 14. The lateral geniculate body in man has the same appearance as in the monkey.

DESCRIPTION OF PLATE IV.

Horizontal section through the hemisphere of the brain of the *monkey*, (Macacus cynomolgus).

C. F., Central Sulcus (Fissure of Rolando).

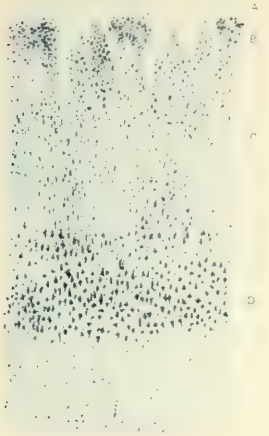
I. P. F., Interparietal Sulcus.

P. O. F., Parieto-occipital fissure.

C., Calcarine fissure.

In front of the central sulcus, is the motor area; behind the central sulcus, up to the parieto-occipital fissure, is the region of the seven-layer type: behind the parieto-occipital fissure is the region of the eight-layer type, or visual cortex.

M. G. SCHLAPP



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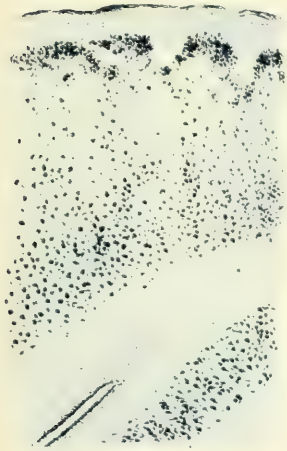

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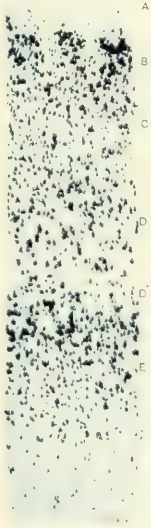

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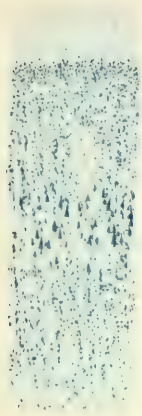

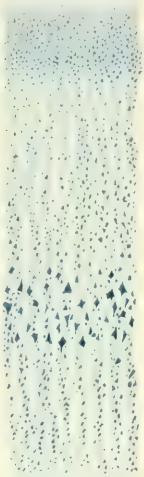

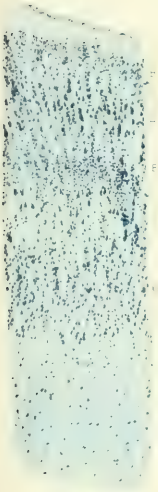

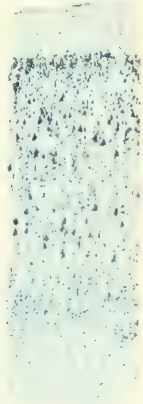

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CORTICAL REGIONS OF THE BRAIN OF A MONKEY.
(*For details refer to explanations of Plates and other Figures.*)

[Reprinted from Journal of Cutaneous and Genito-Urinary Diseases, January, 1902.]

REPORT OF A CASE OF IDIOPATHIC MULTIPLE SARCOMA OF THE SKIN.

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Professor of Skin, Genito-Urinary and Venereal Diseases, Kentucky University Medical Department, Louisville, Ky.

With a Report on the Histopathology,

By Jas. C. Johnston, A.B., M.D.,

CASES of multiple cutaneous sarcomata are relatively rare but in spite of their infrequency quite a number of clinically different varieties have been described. Histologically, however, they show no such great diversity and they may be divided into spindle celled (fusocellular) and round celled (globocellular).

Unna also described a giant cell sarcoma.

Kaposi classes under sarcoid tumors of the skin several apparently distinct disease entities prominent among which is his idiopathic multiple pigment sarcoma. He also describes under type C., in his text book, another variety, referring to it as simply Sarcomatosis Cutis, which differs from the foregoing in many respects. The case I here present exhibits clinically, characteristics of both types. The histological examination, kindly made by Dr. James C. Johnston, shows a greater resemblance to the multiple idiopathic pigment sarcoma than to the other as will be seen by his report. There is an absence though of pigment which in the hemorrhagic variety would naturally be a feature. The patient, however, is of a very fair type, with light blue eyes, reddish hair, and a very white skin, and it may be that a general lack of pigment formation is in a measure responsible for the lighter color of the tumors.

Unna in his "Histopathology of the Skin" makes the following divisions of skin sarcomata. He would first place the so-called

melanotic sarcomata among the carcinomata, claiming that he has found abundant microscopical evidence to justify his doing so. The true sarcomata he divides into two great groups, the solitary, and the multiple. Of the multiple he gives the following five varieties:

1. Sarcoma Multiplex Cutaneum durum album.
2. Sarcoma Multiplex Cutaneum durum pigmentosum.—Piffard.
3. Sarcoma Multiplex Cutaneum Molle.—Neumann.
4. Sarcoma Multiplex Cutaneum gummatodes.—Funk, Hyde.
5. Acrosarcoma Multiplex Cutaneum teleangiectodes.—Hebra, Kaposi.

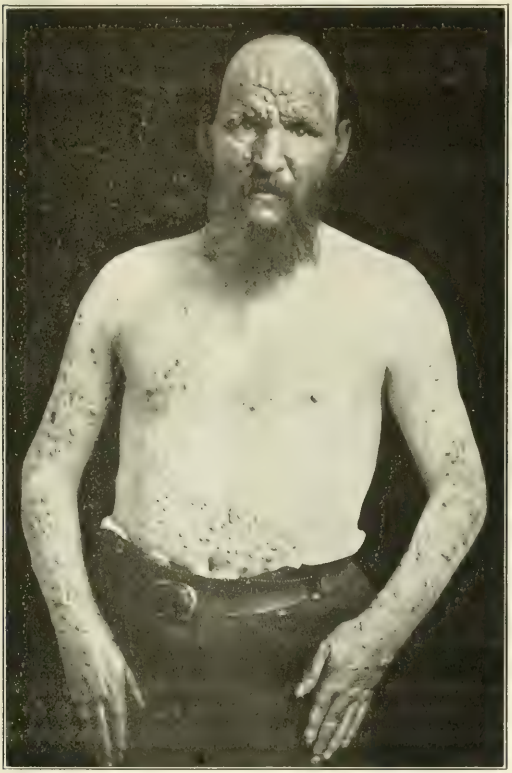
The case here under consideration resembles the description of the first variety closely even to the absence of pigment. Unna regards the fusocellular variety of sarcoma as the main type of the cutis proper since the spindle cells "repeat the normal type of the connective tissue of the skin of adults." A characteristic of the spindle cell variety, as is shown in this case, is the absence of an ulcerative tendency. Ulceration is much more marked in the round cell variety (Type 3 Unna).

Clinical Report: Patient is a Hebrew, born in Russia, and was referred to me by Dr. Leon L. Solomon. He has a good constitution and has always enjoyed good health. He is fairly intelligent and denies ever having had a specific infection. He has been in this country about eight years uninterruptedly and the disease which he is now suffering from developed in 1899. The attack came on immediately after a severe exposure. The patient made a trip to Cincinnati on a boat sleeping in a very warm room. In Cincinnati he slept in a very cold room and on coming home shortly afterward he noticed the tumors for the first time. The eruption commenced first on the extremities, later on extended to the trunk and face. Dr. Solomon tells me that at times several nodules had ulcerated, although the tendency to this seems to be very slight. He complains of muscular pains very much like those of muscular rheumatism. The nodules themselves are painless and devoid of any subjective sensation. The extirpation of one caused profuse bleeding but little pain. In the fall of 1900 he had an acute febrile attack after which he had attacks of dizziness with loss of appetite and pains in knees and legs. In May, 1901, he again had similar pains in lower legs and knees. The patient is a pronounced blonde and has hitherto been entirely free from any eruption of any kind, a point upon which he dwells. Patient perspires very freely and claims that he feels himself weakened thereby. His children are healthy. He is about fifty years of age.

Present state: Health fairly good. No marked deterioration from former standard. Patient at times has fever. As shown in the photo-

graphs the eruption is quite generally distributed and symmetrical. The color of the tumors is a reddish brown somewhat like that of a keloid. A slight purplish tinge is noticed in those on the lower ex-

Fig. 1.



tremities. They are vascular, firm to the touch, elastic with no pain on pressure. The extensor surfaces are more involved than the flexor surfaces. On the forehead the nodules have fused together and give him a leonine expression. The eyelids are much infiltrated. On the

top of the larger tumors there is a distinct condition of telangiectasis. No ulceration anywhere at present. Palms and soles are spared. No lymphatic enlargement anywhere. He exhibits no clinical symptoms that point to leukemia or pseudo-leukemia. Since the advent of warm weather slight hemorrhages have taken place in several of the tumors.

Dr. Solomon in referring this case stated that he had at one time given this patient strontium arsenite, by mouth, with some temporary benefit. He is at present receiving injections of sodium arseniate according to the method of Kaposi. In giving them I notice a general

Fig. 2.

hardness of the skin, as shown by marked resistance to the entrance of the hypodermic needle. This treatment has been used for about a month (0.02 gramme every other day) without appreciable affect on these tumors.

Dec. 7.—The patient took the arsenic injections for about two months. Finding no improvement in that length of time he absented himself. During the last few months he has been without treatment and it is quite apparent that his disease has progressed. The infiltration on the forehead has thickened, and innumerable small nodules

stud his body between the larger ones. The color has also deepened and the tumors look congested. His health is fairly good, he has lost no flesh and has been able to follow his trade of shoemaker. Lymph-nodes nowhere show any involvement. Urine normal, no casts or albumin. He wishes to return to the arsenic treatment as he believes it at least kept the disease in check. There is not the slightest tendency to ulceration or breaking down of tumor tissue.

Histopathology.—The growth which Dr. Koehler sent me measured about three-eighths of an inch in diameter by one-eighth in thickness, but it is apparent from the sections that not all of its mass was included, either peripherally or below. There is a surface erosion at the center. There can be no reasonable doubt that the histological features are the same in all the tumors, although I can not however make a definite statement on the point since I have had only the one for examination. The microscopical picture is a new one in my experience.

The process evidently begins as most skin sarcomas do in the lower reticular layer and upper hypoderm. It never invades the papillary body and seems limited by the lower level of the coil glands beneath. The upper border is flattened and sharply defined (as in fact the periphery is everywhere) by dense collagenous bands running parallel with the periphery. The condensation with the disappearance of the papillæ and interpapillary projections is the result of pressure from the subjacent tumor. The under and lateral borders show lobulated outgrowths into the surrounding skin. In other words, the method of progression is by a gradual pushing aside of the connective tissue as in epithelial neoplasms, rather than the conversion into new growth which is the way with sarcomas even when an attempt is made at encapsulation. Other striking general features are the absence of reactive inflammation of vessel dilatation, and of pigment of any sort.

In the peripheral, presumably newer, portions there is a close packing of spindle cells whose protoplasm stains lightly with basic dyes and whose outlines are indistinct. Their nuclei stain deeply and diffusely like those of young fibroblasts, but, unlike them, are not uniform in size or shape. The latter is greatly varied; some show thickenings like nodes, others are filamentous, some are shaped like spermatozoa and others still are bent at right angles on themselves. Between them, the delicate reticulum of sarcoma is easily brought out by a fresh picric-acid-fuchsin stain. These nuclear distortions can be due to but one thing, outside pressure. The reason for the pressure is found in a totally different type of cell lying between the spindles, even where they are most numerous.

At the growing edge, they are overshadowed by the mass of spindles, but in the central portion, the relative numbers are reversed and the spindle cells almost disappear. The second variety is ovoid or polyhedral, with a protoplasm refractory to most stains (picric acid colors it readily) and oval vesicular nuclei with one or two nucleoli. They lie in nests surrounded by a delicate membrane which does not stain with fuchsin and is not the sarcoma reticulum. With the spindle

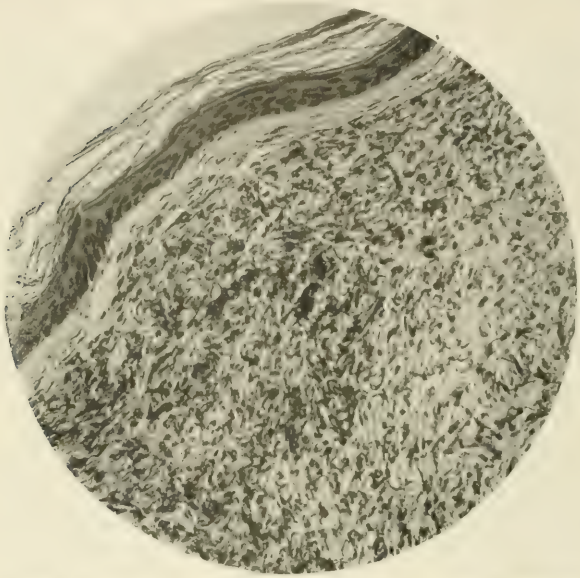


Fig. 3. Periphery of growth showing contorted spindles and disappearance of papillæ and interpapillary projections.

cells, the reticulum disappears. A lumen may be seen in the center of a very few of the nests or whorls which sometimes contains blood. In certain sections, there appeared a giant cell formation with numbers of small round nuclei, but as it often showed both a central lumen and a clearly defined, limiting membrane, I judged it the remains of coil glands caught in the growth as remnants of collagenous material

are ensnared here and there. Mitosis is excessively rare; cell division is direct throughout.

The origin of this second type of cell is not hard to determine. From its morphology and arrangement, it can arise only from proliferative activity in endothelium, from lymphatics and blood capillaries, whether preëxistent or newly formed it is impossible to decide. The whole tumor shows very few patent vessels of any sort but in spite of this, there is no necrosis. Even the englobed tissue remnants are in a perfect state of preservation.

It is a well recognized fact that in sarcoma, particularly of the

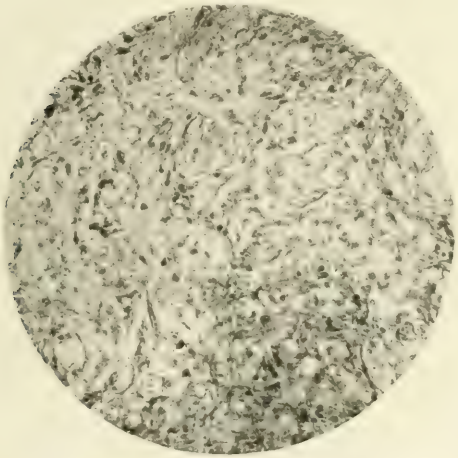


Fig. 4. High power view of an older portion stained with picro-fuchsin to show intercellular reticulum. Spindles have largely disappeared before endothelial new growth.

skin, there is in certain instances a tendency to endothelial proliferation as in Kaposi's idiopathic, pigmented sarcoma and the case which I described in a recent article on this subject. (JOURNAL, July, 1901.) There is no reason to suppose that in a given instance every capillary may not show this proliferation with whorl formation, in the course of time, blotting out almost completely the growth with which it first appeared. If this supposition is correct, the tumor should be classed with sarcomas, although I regard endothelioma as much more nearly

allied to epithelial than to connective tissue new growths both from histogenetic and morphological standpoints. Among the sarcomas, it is clear that its place is with the Sarcoids for these reasons; there is an absence of the appearance of malignancy, such as infiltration of the surrounding skin, of evidence of rapid cell division, of any striking departure from adult cell types, fibroblastic or endothelial (the types are found in pure processes of productive inflammation), and finally, of the absence of metastases, in lymph nodes or viscera. All these peculiarities are characteristic of the class of cutaneous sarcomas which I think we are obliged to make and for which Sarcoid will serve as well as another name. The sarcoids have a cachexia, however, of their own in spite of failure to metastasize. The tumor in question is most closely allied to Kaposi's multiple, pigmented sarcoma which should be called hemorrhagic. Hemorrhage is of course only an accident, though due to peculiarities of structure. It is not present in our case because the vessels are not only fully formed but occluded. The reason for the failure to respond to the use of arsenic is found in the predominance of the endotheliomatous element. Such tumors do not yield to the drug, the fact of its inefficacy furnishing basis for a grave prognosis. As in other endotheliomas, moreover, it is always possible to develop metastasis under irritation or without any appearance of it.

In conclusion, the tumor is a small, spindle-celled sarcoma with which occurs such endothelial proliferation as to strangle the sarcoma element in the older portion. It is free of pigment, hemorrhagic or metabolic, and belongs to the class of Sarcoids, constituting a new type but nearly allied to multiple, hemorrhagic sarcoma. Dr. Koehler is within his rights in classing it clinically with the Unna group he has selected.

My thanks are due Dr. B. H. Buxton for the accompanying microphotographs which admirably illustrate the histological features I have attempted to describe.

Extracted from The American Journal of the Medical Sciences, March, 1903.

A CASE OF BANTI'S DISEASE, WITH DIFFUSE PRODUCTIVE NEPHRITIS.

By Cyrus W. Field, M.D.,
of New York.

(From the Department of Pathology, Cornell University Medical College.)

It is the purpose of this paper to discuss a peculiar and interesting pathological condition, exemplified in a case of advanced productive inflammation of the liver, spleen, and kidneys.

At the outset we are confronted by the difficulty of assigning it to its proper position with complete certainty. There are four pathological conditions closely resembling each other, to three of which this case presents considerable similarity.

1. The condition which is known to the Italian authors as "Banti's cirrhosis" or "Banti's disease."

2. Cirrhosis of the liver, with secondary enlargement of the spleen. In a number of such cases there is also an anæmia of a secondary character.

3. Splenic anæmia, in which we have a marked enlargement of the spleen and a pronounced secondary anæmia.

4. Primary splenomegaly of Bovaird (*epitheliome primitive*, Gaucher)

These conditions will be described with more detail in another portion of this paper.

L. M., housewife, aged thirty-two years.

Family History. Negative. *Personal History.* Measles and scarlet fever when a child. She has had one child, who died at the age of twelve months. No miscarriages. Three years ago she had gastro-intestinal disturbances, and was told that she had a gastric ulcer. She remained in bed at that time for eight months, and was given morphine to dull the pain. She has continued to take the morphine since that time. One year ago œdema of the lower limbs developed, and has persisted. She has used alcohol, but at meals only. No history or signs of syphilis.

Physical Examination. Her general condition is poor. There is marked pallor of the skin and puffiness under the eyes. The mucous membranes are red, and the tongue is large, moist, and clean. There is marked pulsation of the vessels of the neck. There is slight enlargement of the supraclavicular nodes, but no enlargement of any other nodes.

Heart. The apex beat is felt in the fourth interspace, three and one-half inches from the median line, the heart being displaced upward.

There is a systolic murmur heard at the apex and transmitted upward; a systolic murmur heard at the second left interspace and transmitted up to the clavicle; also a systolic murmur heard over the vessels of the neck, of a different character from the above two.

Lungs. At the base of both lungs there is flatness and absence of vocal fremitus. The liver percusses one inch below the free border of the ribs.

Spleen. Nearly filling the left half of the abdomen there is a mass with a smooth surface, which is tender to palpation. The anterior edge of this mass can be traced from a point two and one-half inches from the median line at the costal margin, downward, passing three-quarters of an inch to the left of the umbilicus, reaching the median line at the level of interspinous line of the ilia, and extending two and one-half inches below this line. There is a notch in this mass at a point two inches above and to the left of the umbilicus. The abdomen is distended with fluid. There is marked œdema of the abdominal walls, especially on the left side and of the extremities.

Blood Examination, by Dr. J. W. Coe. Red blood corpuscles, 2,908,000; hæmoglobin, 45 per cent.; leucocytes, 3300. Stained specimens show nothing abnormal.

The urine is yellow, turbid, of specific gravity 1015, and acid reaction. There is a trace of albumin, but no glucose or bile pigment. The sediment is composed of sodium urates and red blood cells.

The temperature ranged between 99° and 100° F., rising just before death to 105° F.

Autopsy, by Dr. A. E. Thayer. There is no rigor. The diaphragm on the right side is at the level of the fourth rib; on the left side, at the fourth space. Pleuræ, old adhesions at both apices. In each pleural cavity there are about 150 c.c. of clear serum. The bronchi contain much pus.

Pericardium. Contains about 100 c.c. of clear serum. The mitral valve is thickened, opaque, and slightly retracted. The chordæ tendineæ are thickened and short. The left auricle is dilated and hypertrophied. The large veins of the thorax are much dilated, and all the blood is fluid.

Peritoneum. There are firm adhesions between the omentum and the pelvic contents on the right side.

Spleen. Weighs 1470 grammes; is firm and dark. On section, shows a marked increase of connective tissue throughout the whole organ. The capsule is thickened, the splenic vein is much dilated. Firm adhesions prevent any movement of the organ.

Pancreas. Appears normal.

Liver. Weighs 3288 grammes; is firm and pale. The capsule is thickened and smooth. On section, this organ shows a great increase of diffuse connective tissue.

Kidneys. The combined weight is 622 grammes. They are large, firm, and pale. The cortex is swollen and anæmic, the markings irregular. The pyramids are congested; there are many punctate hemorrhages on the surface. The ureters and adrenals are normal.

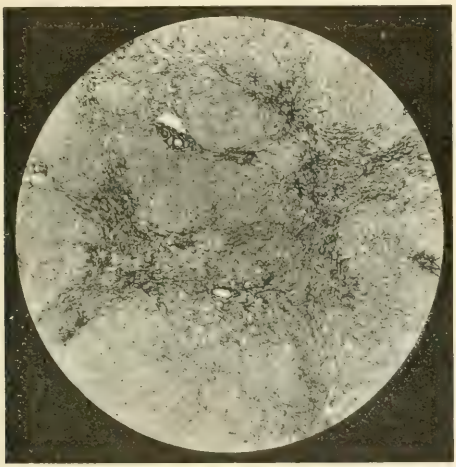
Stomach. Appears to be normal. There are no scars to be found. The common bile-duct is pervious.

Intestines. Peyer's patches are swollen and congested, otherwise normal. The lymph nodes of the mesentery are swollen.

Gross Anatomical Diagnosis. Purulent bronchitis; splenic enlargement; cirrhosis of the liver; nephritis.

Microscopic Examination. Kidneys. Both kidneys show the lesions of chronic interstitial nephritis. The glomeruli appear to be normal in all respects. In some places the tubules are compressed by a diffuse growth of new connective tissue, and in these portions the lining epithelium is degenerated. In the lower portions of the tubules their lumina are seen to be filled with a granular deposit. The bloodvessels in this organ show only slight changes. On staining sections with Weigert's elastic tissue stain it is found that a large proportion of the new connective tissue is composed of elastic fibres.

Fig. 1.

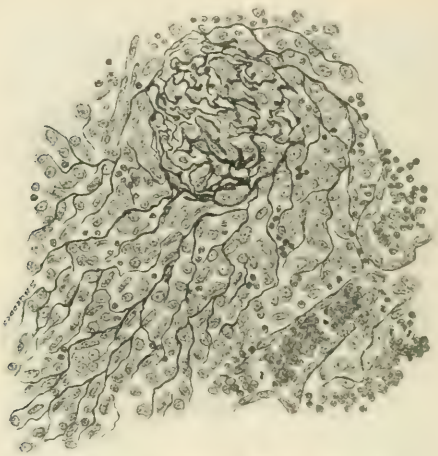


Liver showing diffuse growth of new connective tissue

Sections from different parts of the *liver* show a pretty evenly distributed growth of new connective tissue throughout the organ, not only in the interlobular spaces, but also penetrating the lobules and even separating the individual liver cells. There is no proliferation or new formation of bile ducts, such as is commonly seen in cirrhosis, but the new connective tissue is abundantly infiltrated with small round cells, and in places shows a slight deposit of brownish pigment. Sections stained by Weigert's method show that elastic tissue fibres are present only in their normal situations and in normal amount. The liver cells are in an advanced stage of fatty degeneration. Their nuclei stain faintly, and their protoplasm has an increased affinity for eosin.

Sections of the *spleen* show the same changes throughout the organ. There is a very great increase of the connective tissue, and in a number of sections it is seen that this increase is due to a proliferation of the normal reticulum of the splenic pulp. By the use of Weigert's elastic tissue stain it is found that a great proportion of the connective tissue is made up of elastic fibres. The Malpighian bodies for the most part show a very advanced sclerosis. In fact, some of them are represented by nothing but a mass of connective tissue. Here and there throughout the new connective tissue there is an infiltration with small round cells. In some of the pulp spaces the lining cells seem to have undergone a slight proliferation, some of them lying free in the pulp spaces.

FIG. 2.



Sclerosis of a Malpighian body. Stained same as liver.

We sum up the pathological findings in this case as follows: 1. A marked secondary anæmia. 2. An enlarged spleen, weighing 1470 grammes, its microscopic examination revealing a very marked general hyperplastic interstitial splenitis. 3. A diffuse hypertrophic cirrhosis of the liver of comparatively recent origin. 4. A general productive interstitial nephritis.

1. Of the four conditions to which the present case bears similarity the most noteworthy is Banti's cirrhosis. Banti's disease is characterized by changes in three organs: (1) A gradual enlargement of the spleen; (2) an anæmia of the secondary type, which becomes progressively worse; (3) the later development of a cirrhotic process in the liver. The description which follows is based on Banti's three papers.

The Clinical Picture. Banti divides the course of the disease into three stages. The first he terms the pre-ascitic stage, which dates from the discovery of the first signs of enlargement of the spleen. This enlargement of the spleen may be the only abnormality present for a long time, but sooner or later the blood begins to be affected, and anæmia develops. The red blood cells are seldom under 3,000,000. The hæmoglobin index is slightly below normal. There are no changes in the leucocytes. No nucleated red blood cells are ever found. This stage may last for years, the gravity of the anæmia and the general condition of the patient varying.

The second or intermediate stage is marked by the appearance of gastro-intestinal disturbances. The anæmia becomes more severe, and the quantity of the urine, which until now has been normal, diminishes, and the excretion of urates is increased. This stage may last from a few months to a year.

The commencement of the terminal or "ascitic stage" is marked by the appearance of ascites. The fluid when removed and examined is found to be a transudate. After tapping it reaccumulates very rapidly. The excretion of the urine is markedly lessened. Its specific gravity rises to 1030 to 1040, and it is very rich in urates, and in some cases bile pigment may be present. The patient dies in the course of a few months of general exhaustion, unless of an intercurrent disease.

The Pathological Changes. The Spleen. This organ is found uniformly enlarged. The average weight in ten cases was found to be 1563 grammes, though the range in these ten cases was very wide, as is seen by the following table:

In two cases the spleen weighed	3000 gms.
In two cases the spleen weighed	1500–2000 gms.
In three cases the spleen weighed	1000–1500 gms.
In three cases the spleen weighed	623–1000 gms.

Six hundred and twenty-three grammes was the lowest recorded weight.

The picture under the microscope is the same as that observed in the present case, except in two of Banti's cases, in which we find, besides a great increase in the connective tissue of the organ, a very marked proliferation of the endothelial cells lining the pulp spaces, and the published drawings seem to be like those recently published by Bovaird in his paper entitled "Primary Splenomegaly."

The increase in the connective tissue throughout the whole organ was the only pathological change noted in the other cases. The Malpighian bodies were involved in this sclerotic process to a very great extent.

In some cases they were very markedly atrophied, and no other cellular elements were present except the connective tissue.

The Liver. The lesion in the liver was, in the majority of the cases, an atrophic cirrhosis, though in a number it was of hypertrophic type. In most of the cases the connective tissue was infiltrated with small round cells.

The Kidneys. In only two cases was the condition of the kidneys mentioned, except in a most superficial way. In the two cases in which a definite statement was made it was only said that a nephritis was present, and so I am unable to state the exact type of nephritis that occurred in these two cases. Judging from the clinical histories of the other cases it would seem that there must have been a nephritis present in a number of them.

The bone-marrow was normal in all except three cases, and in these it was stated that it had reverted to the fœtal type.

To sum up the pathological conditions present in this so-called "Banti's cirrhosis," we have: 1. An anæmia of a secondary type. 2. A very great enlargement of the spleen, due to a hypertrophy of all the cellular elements, but more especially of the connective tissue. 3. A cirrhosis of the liver, but not of a constant type. 4. An inflammation of the kidneys in a number of the reported cases.

2. *Cirrhosis of the Liver, with Secondary Enlargement of the Spleen.* The second group of cases comes under this head. We often see, in long-standing cases of cirrhosis of the liver, a more or less enlarged spleen and also very often a marked secondary anæmia. Now, in regard to the enlargement of the spleen secondary to cirrhosis of the liver I have searched the literature to find some definite figures as to the size it may reach, but have been unable to find any. It would seem, though, from the autopsy records to which I have had access, and from statements of experienced pathologists, that the spleen in this condition never reaches the size and weight of that organ as found in "Banti's disease," splenic anæmia, or primary splenomegaly.

3. *Splenic Anæmia.* The only point of dissimilarity between this condition and "Banti's disease" is that in the former the liver is normal, while in the latter that organ is found to be cirrhotic.

4. *Primary Splenomegaly.* This term should be reserved for those cases which present a splenic lesion similar to that described by Bovaird.[5] In his case the splenic enlargement was due to a very great proliferation of the endothelial cells lining the pulp spaces, and the increase in the connective tissue elements was but nominal. The clinical picture as appearing in his description is very like that of the other conditions.

Differential Diagnosis. We may differentiate between cirrhosis of the liver with secondary enlargement of the spleen and the three other conditions—namely, "Banti's disease," splenic anæmia, and primary splenomegaly—by the following facts: 1. The spleen in cirrhosis of

the liver never attains the size that it does in the three other conditions. 2. The anæmia in cirrhosis is apt to be less severe than in the other conditions. 3. In cirrhosis the splenic enlargement is secondary, while in the other conditions it is the primary lesion.

This last point is of very slight diagnostic value, as the vast majority of the cases is not seen until too late a stage of the disease, and, as Osler[16] has pointed out, we cannot be sure of the presence or absence during life of a cirrhotic process in the liver.

The diagnosis between splenic anæmia and primary splenomegaly can only be made by microscopic examination of the spleen, as there is no difference in the conditions except that in splenic anæmia the increase in size of the organ is due to a general hyperplasia of all the cellular elements, but more especially of the connective tissue; whereas in primary splenomegaly the increase is due to a very great proliferation of the endothelial cells lining the pulp spaces, with only a moderate increase in the connective tissue.

The only point of difference between "Banti's disease" and splenic anæmia is that in the latter there is no cirrhosis of the liver; therefore we may say that "Banti's disease" is splenic anæmia plus a cirrhosis of the liver.

Conclusions. 1. There are four conditions, more or less closely related, which may be distinguished as follows: Enlargement of the spleen with anæmia and the later development of a cirrhotic process in the liver may be separated from cirrhosis of the liver, with secondary enlargement of the spleen, by the fact that in the latter condition the spleen never attains the size that it does in the former. Both these conditions may be separated from splenic anæmia and primary splenomegaly by the presence of the cirrhosis of the liver, while these two latter conditions may only be differentiated from one another after a microscopic examination of the spleen.

2. The case here reported should be placed among those cases termed "Banti's disease" for three reasons: (*a*) The very great enlargement of the spleen; (*b*) the presence of a cirrhotic process in the liver; (*c*) the fact that the enlargement of the spleen was due to a general hyperplasia, but more especially of the connective tissue.

3. The present case, exhibiting an advanced chronic productive nephritis, somewhat extends the scope of the pathological anatomy of Banti's disease.

Speculation as to the etiology of the disease seems at present premature, but the increasing recognition of the occurrence of transitional cases connecting together Banti's disease, splenic anæmia, and diffuse cirrhosis of the liver suggests an origin from a common cause emanating from the gastro-intestinal tract.

Finally, I would thank Prof. James Ewing for placing this case at

my disposal, and for many kind suggestions during the preparation of this paper. For the photomicrographs I am indebted to Dr. B. H. Buxton.

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ENZYMES IN TUMORS

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ENZYMES IN TUMORS.[1]

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The following is a preliminary report on a line of experimental work undertaken in connection with the Huntington fund for cancer research, and carried out in the pathological laboratory of Cornell Medical College.

My best thanks are due to many of my confrères, more particularly to Dr. Carl Beck, for material kindly provided.

I am also greatly indebted to Professors Green, of Cambridge, and Beijerinck, of Delft, both of whom very kindly gave me most valuable instruction last summer in the methods of testing for enzymes.

The object of the work is to endeavor to determine:

(1.) The nature of any enzymes present in malignant tumors.

(2.) If any particular enzymes are constantly present.

(3.) If so, are they in any way different to those found in normal tissues.

The enzymes in question would, of course, be strictly intracellular as opposed to the extracellular enzymes of the digestive tract. The extracellular enzymes have long been known and very closely studied, but until comparatively recent years, ferment action occurring in the animal cell itself was ascribed to vital action of the protoplasm. It was supposed that in this respect there is a difference between vegetable and animal enzymes: the former acting in the cell itself, whilst the latter are poured out into the digestive tract to do their work outside of the actual organism.

It gradually came to be recognized, however, that from animal cells — cells, moreover, which are not concerned in the manufacture of extracellular enzymes there can be extracted by maceration in water certain soluble principles, which can

[1] Received for publication April 6, 1903.

exert various ferment actions altogether apart from the life of the cell.

By grinding yeast cells to powder and pressing out the juice, Buchner was able to extract zymase, the principle which converts glucose into alcohol and carbon dioxide. It has been found impossible by simple maceration of yeast to obtain any such ferment apart from the living cell. Experiments on the lines of mechanically tearing cells to pieces in various ways are being systematically carried on at the Jenner Institute in London by MacFadyean and Rowland, but it still remains an open question whether animal cells can be got to yield by such methods any enzymes which cannot be extracted by simple maceration.

The subject of intracellular ferments for normal tissues has been worked over pretty thoroughly in recent years, but as applied to pathological tissues has received but little attention. Petry[24] found that carcinoma tissue very readily undergoes autolysis, with increase of non-coagulable proteids and formation of leucin, tyrosin, hexon and purin bases at the expense of the more complex proteids coagulable by heat. The autolysis is due to the presence of a proteolytic ferment, the products corresponding to those found by Salkowski and others in autolysis of normal organs.

Emerson[10] in a study on the influence of carcinoma of the stomach on digestion, comes to the conclusion that in carcinomatous tissue there exist proteolytic ferments which carry the digestion beyond the albumose and peptone stages. Such ferments dissolved out from the ulcerated surface of carcinoma of the stomach accelerate the action of the pepsin, and their ultimate products, diamido bodies, and so forth, enter into loose combination with the HCl. In this way he accounts for the absence of free HCl in such cases. There are some weak points in his arguments, and the work needs confirmation, which Emerson says it is receiving at the hands of Dr. Falta, of Basel.

EXPERIMENTAL METHODS.

The first step to be taken was to test a number of malignant tumors to see what enzymes they contain. Since it is not always easy to obtain cancer material in sufficient quantity to make filtered extracts and test for enzymes in solution, an attempt was made to demonstrate their presence by means of diffusion in agar plates.

For this the methods adopted by Beijerinck[2] and Eijckman[8] for demonstration of vegetable and bacterial enzymes have been utilized and modified to suit requirements. It is fully realized, however, that the results obtained require checking and confirmation by means of extracts prepared and tested in the usual way, and the intention is to extend the work in this direction. If the method of diffusion in agar plates proves reliable, as there seems little reason to doubt will be the case, it will certainly be a convenient one to adopt, since very small amounts of tissue are required, and the method is simplicity itself. To quantitative determinations it is obvious that it can never be applied. At the best only rough comparisons can be made.

Whatever the method adopted, however, there will always be two great disturbing elements in examination of the cell products of tumors:

(1.) The presence of blood from which the tissues cannot be freed by the methods used in animal experiment.

(2.) The presence, especially in carcinoma, of tissue which merely represents a productive inflammatory reaction, and is not, properly speaking, a part of the tumor itself. Fully formed connective tissue contains little or no active ferment, but young connective tissue and leucocytes will always have to be reckoned with.

The methods of procedure were as follows: The tumor, or part of it, freed as far as possible from other tissues was weighed and chopped fine. About an equal amount of pumice stone was added, and the whole pounded as fine as possible in a mortar. Enough fifty per cent glycerin in water was then added to bring the mass to the consistence of

a soft paste or pulp. Vernon[80] found fifty per cent glycerin the best extractive for enzymes.

The pulp was then divided into two parts, and put into two sterile bottles labelled A and B: B was boiled for twenty minutes, and a little more fifty per cent glycerin was added to bring it again to its original consistence. Twenty or thirty grams of each were found to be sufficient, and for some small tumors a good deal less had to be put up with. A little chloroform was added to each, stirred well in, and renewed from time to time as found necessary.

The tumor was at once tested bacteriologically. A loopful of the pulp was put into a melted agar tube and plated out. It may be mentioned once for all that the tumors tabulated here were practically sterile. There were in some cases a few colonies on the plates, but never more than could be accounted for by unavoidable contamination during manipulation.

After keeping for two or three weeks, the pulp became absolutely sterile, except for the occasional presence of a few spores belonging to the mesentericus or hay bacillus group. It may be taken for granted, then, that there were no enzymes due to previous infection by bacteria present in any of the tumors.

The agar used as a basis for the plates was made up to two per cent with tap water, boiled till dissolved, cleared by prolonged boiling with white of egg, and filtered through cloth and absorbent cotton. The agar was distributed into small flasks, 100 cc. in each, more or less, as required, and sterilized.

When needed for use the agar was melted and a little thymol added whilst hot, so that it would dissolve thoroughly. The agar was then cooled down gradually to 50° C. or 60° C.; the substance needed for the reaction added, and as soon as this was thoroughly mixed or dissolved, the agar was poured into Petri dishes, about 15 cc. in each, and allowed to set.

A small spoonful, about ½ cc., of the pulp A and another of B were put on the surface of the agar, spread out in a

circle of 20 mm. diameter, and the plate kept in an air-tight incubator at 40° C., exposed to vapor of chloroform water. In spite of the thymol and chloroform, it was found necessary to conduct the operations as aseptically as possible, since some spore-bearing bacilli of the mesentericus or hay bacillus groups would occasionally, though not often, grow on the plates after two or three days. This was not of much consequence, however, since the observations on the enzymes seldom extended over more than forty-eight hours. Fresh milk could not be used for the milk agar plates, or there would be countless colonies in forty-eight hours. There is one advantage in using agar plates, and that is, that any contamination by bacteria can be at once detected.

EXPERIMENTS.

1. *Proteolytic enzymes.*

(*a.*) *Egg albumen.* Suspension 5 per cent of white of egg in agar. Slightly opalescent.

This has been found very useful for demonstration of bacterial enzymes. An old culture of anthrax, for example, together with some of the agar on which it has been growing, is smeared over the surface. In twenty-four to forty-eight hours a clear ring of 10–20 mm. all around the smear can be seen, and the effect is heightened by heating over a flame. The egg albumen coagulates, and the agar becomes quite opaque, except for the ring around the anthrax culture. Growth has been prevented by the thymol and chloroform, so that the change must have been due to diffusion of enzymes previously secreted.

This method applied to tumors, however, led to no positive results. In none of them could proteolytic enzymes acting on egg albumen be demonstrated.

Hedin & Rowland [15] found that intracellular proteolytic enzymes of the spleen were most active in slightly acid solutions. In view of this, and of Emerson's experiments already mentioned, egg albumen plates with .35 per cent HCl were used in some cases as well as the neutral ones, but here also the results were negative.

(*b.*) *Milk.* Agar, 90 per cent; sterilized milk, 10 per cent.

The milk plates treated with anthrax, as already described for egg albumen, show in twenty-four hours a ring of coagulation (rennet), and later on an inner clear ring due to digestion of the coagulated casein by a proteolytic enzyme. The results obtained with tumors were uneven:

Some showed no change;

Others clearing without previous coagulation;

One coagulation and no subsequent clearing;

Whilst some showed both in the clearest possible way.
None of the boiled controls had any effect on the milk.

(*c.*) *Liquefaction of gelatine.* Ten per cent water gelatine with a little thymol into Petri dishes kept at room temperature.

Here again the results were uneven. With some tumors there was liquefaction, more or less rapid, and with others none. In no case did the boiled controls have any effect on the gelatine.

Eijckman[8] found that among the bacteria those which liquefy gelatine also digest casein and vice versâ. My experiences coincide with his so far as bacteria go, but this was not always found to be the case with the tumors. No. 28, for instance, had no effect on gelatine, but coagulated and then digested the milk in a very marked way.

(*d.*) *Hemolysins.* Several experiments were made with agar + .7 per cent NaCl, in which a little defibrinated blood was suspended. In this the blood corpuscles remain intact, but are dissolved by thymol or chloroform vapor. Antiseptics, therefore, could not be used, and it has hitherto been found impossible to avoid some contamination of the plates so that the results have been negative, although there were indications of hemolysins in some instances.

(*e.*) *Fibrin.* Pounded fine and suspended in agar was never affected in the least.

(*f.*) *Serum* Coagulated in Petri dishes was not liquefied, but only a few of the tumors were tested in this way.

The experiments with proteolytic enzymes then afforded uncertain results. The softer and more cellular tumors one would expect to contain more than the denser, more fibrous ones, but this does not always appear to be the case, although it usually is.

Petry, whose experiments on autolysis of carcinoma tissue have already been mentioned, has been quoted as saying that such autolysis proceeds more quickly than with normal tissues. But he actually only made comparisons with normal breast tissue, which is relatively poor in cells, and there seems so far no reason to suppose that carcinoma tissue undergoes autolysis more rapidly than normal highly cellular organs, such as liver.

Jacoby[17] has recently advanced the opinion that the proteolytic intracellular ferments of the various organs are to some extent specific. He found at any rate that ferments in liver extract, which have the power of reducing the amount of proteid coagulable by heat in liver tissue, have no effect

on the coagulable proteids of lung tissue, although they can attack the albumoses in both instances. He points out that the coagulable proteids of lung and liver would never come in contact with each other, but the albumoses from the lung might reach the liver and other organs by way of the circulation, as has been recently shown by Embden and Knoop[9] and Langstein.[10] The former then have developed specificity, and can only be attacked by specific enzymes,* whilst the albumoses are general, and can be acted upon by enzymes common to all organs. Jacoby finds an analogy to this in the action of the complements, some of which are strictly specific, whilst others have a more general character.

Some such specificity of the proteid and enzyme molecules may, perhaps, account for the egg albumen in the agar plates always remaining untouched, whilst the non-coagulable casein and the still simpler gelatine were often digested.

This hypothesis of hyper-specificity, as it may be called, for proteolytic ferments does not, of course, apply to those of the digestive tract, but only to such as act intracellularly.

It seems, however, more probable that the proteolytic enzyme of the tumors is allied to the erepsin of Cohnheim (Zeit. Phys. Chem., Vols. 33, 35, 36, 1901 and 1902), found by him in the secretions, but more particularly in the mucous membrane of the small intestine. Erepsin cannot attack egg albumen or fibrin, but can actively reduce, not only albumoses and peptones, but also casein to crystallizable products; leucin, tyrosin, etc.

Cohnheim maintains that erepsin is an intracellular ferment which attacks the peptones at the moment of their resorption by the cells of the mucous membrane.

Amylase. — Two kinds of plates were prepared:

(1.) Agar, 100 c.c. + soluble starch .5 grams.

(2.) Agar, 100 c.c. + glycogen .25 grams.

After two days the plates were flooded with HCl 3.5 per cent for a minute or so to remove any traces of alkalinity

* Bokorny[3] considers that in the present state of our knowledge we must assume a special plasma for each function of the cell, and emphasizes the intimate relations of the protoplasm of a cell and its enzyme.

which would interfere with the subsequent reaction. The HCl was then poured off and diluted Lugol solution was poured over the surface. The starch agar turns deep blue and the glycogen brown, except where there has been some conversion into sugar.

In every case there was some conversion shown by a clear ring around A, whilst B always remained unchanged. In the tables the width of the rings is marked in millimeters in each case.

Glycogen has often been demonstrated in tumor cells by micro-chemical methods. Lubarsch[23] could only find it in sixty-two out of four hundred and eighty tumors, but Brault[4] claims that it can always be found, if small pieces of the tumor tissue are taken quite fresh and put directly into absolute alcohol, so that the glycogen has no chance to dissolve out. The more malignant the tumor, the more glycogen it contains, and prognosis can be based on the amount observable.

A reversing action for some enzymes has been demonstrated. Croft Hill showed that if maltase be added to a concentrated solution of glucose some of the latter is synthesized by dehydration into maltose.

Hanriot,[14] Loevenhardt,[22] and others have shown that lipase has a reversing action and can either synthesize or analyze fats in such a way as to maintain the necessary equilibrium in the various cells of the organism.

Cremer,[6] on adding glucose to Buchner's pressed yeast extract, found traces of glycogen were formed, but it cannot yet be considered proved that amylase is able to exert a reversing action.

That the glycogen in tumor cells is synthesized "in sutu" and not brought in as glycogen, there can be little doubt, and it is significant to find amylase so constantly present in tumor tissue. A future study will be to compare the amounts of glycogen and amylase found in tumors and see if they bear any relations to each other. Up to now the amount of amylase present, so far as can be roughly judged by width in millimeters of starch converted, does not seem to vary directly

with the malignancy. The very malignant tumors of the liver contained comparatively little amylase. This again seems significant and worth further investigation, since the adult liver contains glycogen, whilst it is absent in the embryonic liver. In other tissues the reverse obtains, with the exception of the muscles which do not enter into the question so far as malignant tumors are concerned.

Lipase. — For demonstration of this in the blood and other organs, Hanriot used solutions of butyrin, and Loevenhardt of ethylbutyrate, with positive results in both instances, so far as the blood is concerned.

Doyen and Morel,[7] using emulsions of olive oil, could not confirm Hanriot's experiments, but concluded that blood contains no lipase, and that the formation of acid in Hanriot's solutions was due to bacterial growth. Arthus [1] found a ferment in blood which converts butyrin into glycerin and butyric acid, but has no effect on vegetable or animal fats. He calls it butyrase, distinguishing it from true lipase. It is a question, however, if the action be not one of degree rather than of kind. The lipase present may be sufficiently concentrated to convert butyrin, but not the more stable compounds of the higher fatty acids. An analogous instance may be found in the metacasein reaction. Roberts [26] showed that milk treated with pancreatic extract does not curdle at room temperature, but will do so on boiling. He concluded that he had demonstrated a milk-curdling ferment of a different nature to true rennet, but it is now held that it is only a question of the amount of rennet and the conditions under which it acts.[12]

The method adopted here was to mix the fatty substance with litmus agar just sufficiently alkalinized to turn the litmus blue. The lipase diffuses out into the medium, converting the fat into a fatty acid and something else; the litmus turning red on account of the acid liberated.

Neither ethylbutyrate nor oil emulsion were found to give satisfactory results; only traces of acid being occasionally formed, and in the great majority of instances none at all. It appears as if the lipase present is not strong enough to

decompose the substances under the conditions offered, but the more labile butyrin (one per cent in agar) always yields positive results. In an hour or so there is distinct reddening all round A, and in five hours a red ring, varying from one millimeter in some instances to five or six in others. The reddening is progressive, spreading all over the A side of the plate in twenty-four hours.

Whether it be true lipase or not, there is certainly something in the tumors which can rapidly convert butyrin into glycerin and butyric acid, and this power is destroyed on boiling, since B never showed any traces of reddening.

Bacterial growth was excluded, and the original reaction of the pulp was either neutral or so very faintly acid to litmus that it had no visible effect on plain litmus agar plate.

It is very fascinating to watch the progressive reddening, but it must be confessed that the value of the reaction for tumors will probably prove to be nil, since normal rabbit organs, and more particularly the blood, were also found to convert the butyrin in every instance.

Suspensions of butter in agar, about two per cent, were tried for micro-organisms. No bacteria were found which could split the butter, but aspergillus niger, which is known to secrete lipase,[5] reddens the litmus all around the growth in forty-eight hours.

On applying this to the tumors it was found that in some instances there was slight reddening, but the results were uncertain and not worth recording.

Oxidases. — Animal tissues are able to readily oxidize many substances which in the chemical laboratory can only be oxidized very slowly at body temperature, or rapidly at much higher temperature. Until recently this power of accelerating oxidation was attributed to vital energy, but it is now known that it is due, partially if not entirely,*

* Jacoby[10] points out there are many substances oxidizable in the body which ferments will not oxidize. It may, however, be only a question of time before ferments for these are discovered.

to soluble oxidizing ferments, oxidases, which can be extracted from the cell, and exert their influence altogether apart from it. The oxidases in the biological seem to play the part of heat in the chemical world.

Again there are substances, such as oxalic acid,[25] easily oxidizable by chemical methods, which pass through the body unchanged, and are therefore not affected by the oxidases.

The oxidases, then, can exert a selective action, presumably oxidizing such substances as in their oxidized condition will be of use to, or less harmful to, the cell, whilst those which are indifferent or naturally foreign to the organism are not affected.

The individual oxidases do not appear to possess specificity. Those which respond to one reaction will, as a rule, respond to others, indicating that they can oxidize more than one particular substance, but there are certainly different kinds. A ferment can be extracted, for instance, from liver tissue which does not give the guaiac reaction, but can oxidize salicyl aldehyde to salicylic acid.[29]

The oxidases, like other ferments, are destroyed by boiling. There are several reactions by which their presence may by recognized.

1. The Guaiac test. (*a.*) To a little distilled water in a tilted porcelain dish a saturated alcoholic solution of gum guaiac is added to the point of a milky emulsion.

A small quantity of the tumor pulp is placed at the margin of the fluid, and a blue color due to oxidation of the guaiaconic acid contained in the guaiac, would show the presence of oxidase. In no case did the blue color appear. Oxidases which turn guaiac blue then were uniformly absent, nor were they ever found in normal organs, human or animal. The latter observations agree with those of Rosell,[27] who in normal organs never found oxidases turning guaiac blue. (*b.*) On adding a little H_2O_2 to the above after thirty minutes or so, a blue color always appeared, more or less rapidly diffusing throughout the emulsion. Peroxidases, therefore, were always present, and the same holds good for

normal tissues. Blood, as is so well known, gives the reaction very readily, and its presence in the tumors no doubt accelerates the action, though Jacoby [18] says tissue freed from blood is also active.

This property of turning guaiac blue in the presence of H_2O_2 was greatly weakened, though not entirely lost, on boiling, so that there must be something present besides the peroxidases which can oxidize the guaiac. Schaer,[28] indeed, found that there are a great many such substances.

H_2O_2 ferment (Catalase). All the tumors decomposed H_2O_2 with release of oxygen, and this appears to be a property of all living protoplasm. Boiling invariably destroyed the activity.

This ferment is probably identical with peroxidase, the liberated oxygen in the presence of guaiac having the power of oxidizing it.

Indo-phenol reaction. — Freshly dissolved dimethylparaphenylendiamin and *a* naphthol were mixed in equal parts and 6–8 cc. added to each 100 cc. of agar, at once plated out, and the tumor pulp placed on the surface. In twenty-four hours such plates have become pale blue all over (indophenol), but where the reaction has been accelerated and intensified by oxidizing ferments, as is always the case with a slice of potato, there is very distinct deep blue ring all round the substance. The tumors never afforded such clear deep blue rings as the potato, and the reaction often failed to materialize at all, so that the results so far obtained do not seem to point to any conclusion. Rosell observed this reaction with some normal organs and failed with others.

Lillie by micro-chemical methods demonstrated the indophenol reaction in leucocytes and other cells, the blue color appearing most intense in the neighborhood of the nuclei.

Hydrokinone reaction. — Agar, 100 cc.; hydrokinone, 1 gram. In solutions containing oxidases the hydrokinone is gradually oxidized to the dark reddish quinone and after a

	Tumours.	Proteolytic.						Amylase.				Lipase.		Oxidase.			
		Milk.				Gelatin.		Starch.		Glycogen.		Butyrin.		Hydrokinone.		Indophenol.	
		Coag.	mm.	Clear	mm.	Liq.		Clear.	mm.	Clear.	mm.	Red.	mm.	Dark.	mm.	Blue.	mm.
1.	Carcinoma breast scirrhous					+		+	5	+	2			+			
2.	" "	—		—		—		+	5	+	3	+	10	+	2–3		
3.	" " scirrhous	—		—		—		+	5	+	2–3	+	10	+	2		
4.	" " cellular	—		—		—		+	8	+	6	+	10	+	slight	—	
5.	" lymph nodes of No. 4			+	trace	—		+	7	+	4	+	12	+	slight		
6.	" breast scirrhous	+	4	+	2	+	slight	+	5	+	3	+	10	—		—	
7.	" "			+	slight	+	saght	+	3–4	+	slight	+	3–4	+	trace	trace	
8.	" " scirrhous			+	3–4	—		+	10			+	6	+	trace	+	
9.	" " "	+	1	+	4	+	slight	+	10	+	5	+	10				
10.	" " cellular	+	5	+	3	+	much	+	8			+	10			+	2–3
11.	" " scirrhous	—	—	—		+		+	4	+	3	+	12	+	5		
12.	" liver			+	3	— ?		+	5	— ?		+	15	+	5	+	?
13.	" "	+	3	+	3	—		+	5–6	— ?		+	20	+	slight		

14.	" "	+	5	+	3	—	.....	+	5	+	2–3	+	much	+	5	+	slight
15.	" "	+	5	+	2	+	much	+	5	+	little	+	much	+	4–5	+	
16.	" "	+	5	+	5	+?	.....	+	4–5	.....	.....	+	much	.....	.....	+?	
17.	" bladder	—	.....	—?	.....	—	.....	+	5	.....	.....	+	15	+	slight		
18.	Epithelioma tongue					—	.....	+	.....	+	.....	.....	.....	—			
19.	" inf. maxilla						.....	+	5–6	+	5	+					
20.	" same as 19	—	.....	+	5	+	much	+	7	+	5	+	10	+	5	—	
21.	" lip					—	.....	+	5	+	2	+	8	—			
22.	"	—	.....	+	4	+	.....	+	4	+	4	+	12	+	2–5	+	slight
23.	" orbit	—	.....	+?	2–4	+	much	+	7	+	2	+	8	+	3–4	+	2–3
24.	Sarcoma melano	—	.....	—	.....	+	.....	.....	.....	+	2–3	.....	.....	+			
25.	Sarcoma spindle (outer part)		.....	+	trace	—	.....	+	slight	+	slight	+	8	+	3–4	+	1
26.	" " (inner part)		.....	+	slight	—	.....	+	slight	+	?	+	10	+	4	+	1
27.	Sarcoma angio	+	6–7	.....	.....	+	much	+	5	.....	.....	+	much	—	.....	+	slight
28.	" pectoral	+	1	+	3	—	.....	+	6	.....	.....	+	10	+	2–5	—	
29.	" periosteal	—	.....	—	.....	—	.....	+	7	+	4	+	14				
30.	Tumor of parotid	—		—		—		+	6	+	7	+	10	+	2		

time green crystals of quinhydrone separate out. On agar plates the green crystals were never observed, but potato in a few hours becomes surrounded by a dark ring. The tumors never showed this so clearly as the potato, and the results were irregular and unsatisfactory.

Besides these, a few experiments were made on agar plates with indican, changed to indoxyl by indiesac,[2] the indoxyl being oxidized by free oxygen to indigo blue; and tyrosin, blackened by tyrosinase,[11] but the results were entirely negative.

Experiments with oxydases have, therefore, led to no particular results, but so little is known of these ferments and the necessary conditions for their action, that this was only to be expected at first.

The idea of suboxidation as a cause of tumor formation seems to be gaining ground, but so far is purely speculative, having no experimental basis on which to rest. With this idea in mind, however, an extended study of the oxidizing ferments found in tumors, and a comparison of them with those of normal tissues, may help to confirm or overthrow the hypothesis.

The accompanying tables show in detail the results obtained. Tumor pulps kept for a month or two months, and tested a second and third time, never showed more than trifling variations from the primary observations either in quantity or in quality of enzymes, so that their variations have not been taken into account in making up the tables.

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MYCOTIC ENZYMES.

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In dealing with ferments, a distinction is usually drawn between unorganized soluble ferments—enzymes secreted by multicellular plants and animals and organized living ferments, the mycetes (molds, yeasts, and bacteria)—which were supposed at one time to effect changes in organic matter by direct protoplasmic action only.

This, however, is a distinction without a difference, since it has more recently been shown that those nutritive substances which are not directly assimilable by higher organisms cannot be directly assimilated by the mycetes either, and that these latter also convert non-assimilable into assimilable substances by means of enzymes. The only difference is that among the higher organisms certain cells are specialized for the production of enzymes, while with the mycetes each cell has to do all its own work.

Taking the carbohydrates and proteids as examples, we find that these must be simplified to precisely the same stage, both for multicellular and unicellular organisms, before they are directly assimilable.

The more complex carbohydrates, the polysaccharids $(C_{12}H_{20}O_{10})$ n., such as starch, glycogen, and inulin, and the disaccharids $(C_{12}H_{22}O_{11})$, such as cane sugar, lactose, and maltose must be converted into monosaccharids $(C_6H_{12}O_6)$, such as glucose and fructose, while the proteids, both insoluble, and such as enter into colloidal solution, as for example albumin, must be converted into peptones which enter into true solution.

The monosaccharids and peptones alone then among these two classes of substances can be directly assimilated by the protoplasm of living cells whether the cells lead an independent existence or form a part of a complex.

Any further breaking down of these substances by the direct action of the protoplasm may be called catalysis, though it seems quite possible that even the catalytic action may in reality be effected by means of enzymes working intracellularly, since Buchner has shown this to be the case with yeast, which effects the

conversion of glucose into alcohol and carbon dioxid by means of an enzyme (zymase), existing in such close connection with the protoplasm of the cell that it can only be extracted by literally tearing the cell to pieces, and after extraction, *i. e.*, separation from the protoplasm, very quickly loses its activity.

Zymase, then, unlike the enzymes known hitherto, is not naturally diffusible, and it seems quite probable that in intracellular juices other nondiffusible enzymes, which cannot be extracted by simple maceration, may be demonstrated.

Nondiffusible enzymes, such as zymase, may be designated as intracellular, but the diffusible enzymes may act intracellularly, as, for example, the amylase of the liver.

In connection with intracellular ferment action may be mentioned certain diffusible enzymes —oxidases— which have the power of oxidizing various substances less complex than monosaccharids and peptones. For example Spitzer demonstrated an oxidase in liver and spleen pulp, which oxidized hypoxanthin (oxypurin) to xanthin (dioxypurin), and finally to uric acid (trioxypurin).

The oxidases are very widely distributed both in the animal and vegetable kingdoms, and in one form or another are probably a constant accompaniment of living protoplasm. It may be then that protoplasm never exerts a direct catalytic action, but effects all its breaking down by means of enzymes which it secretes, reserving for itself a synthetic action only. Oppenheimer (p. 249) remarks, "A ferment can indeed split or oxidize, but can never effect syntheses."

Even this assertion, however, may have to be modified since Croft Hill and others have recently shown for a few hydrolyzing enzymes that they can exert a reversing action, under certain circumstances, and hydrate or dehydrate (synthesis), according to the nature of their environment. Hanriot, indeed, goes so far as to suggest that diffusible enzymes acting intracellularly, as lipase in fat cells, and amylase in liver cells, play the part of regulators, keeping certain constituents of the cells in constant proportion by splitting or synthesising as occasion arises.

However, in the present state of our knowledge we must allow to protoplasm the power of catalytic action, beginning where accurate knowledge of enzymes leaves off, and for all practical purposes this knowledge ceases with monosaccharids and peptones so far as the carbohydrates and proteids are concerned.

The action of the enzymes on the more complex carbohydrates is one of hydration:

$$\underset{\text{Polysaccharid}}{(C_{12}H_{20}O_{10})n.} + nH_2O = n\underset{\text{Disaccharid}}{C_{12}H_{22}O_{11}}$$

$$\underset{\text{Disaccharid}}{C_{12}H_{22}O_{11}} + H_2O = 2\underset{\text{Monosaccharid}}{C_6H_{12}O_6}$$

Amylase.—The enzymes acting on the polysaccharids, starch and glycogen, are called collectively amylase. Whether they are identical, from whatever source they may be derived is still uncertain, but their action on starch and glycogen appears to be practically similar in all cases. The first step is to change the insoluble into soluble starch, the soluble starch being then hydrated to maltose (disaccharid); certain intermediate substances, the dextrins, being formed at the same time. It is supposed by Brown and Morris,[7] although Brown[8] appears to have more recently modified his views, that there are numerous varieties of dextrines, but however this may be, they can at any rate be divided into two distinct groups, the erythrodextrins which turn red, and the achroodextrins or leukodextrins, which remain colorless on the addition of iodin in the shape of diluted Lugol solution. The steps then in the process are:

		Solution.*	With iodin.
1	Starch	Insoluble.	Blue.
2	Soluble starch	Colloidal.	Blue.
3	(a) Erythrodextrins	Slightly colloidal.	Red.
	(b) Leukodextrin		No change.
4	Maltose	True.	No change.

The changes in glycogen conversion are analogous to, though perhaps not absolutely identical with, those of starch.

According to Duclaux, amylase contains two distinct enzymes: (1) Amylase which liquefies the starch, *i. e.*, reduces the solution aggregates without affecting the individual molecules, bringing it down to the dextrine stage, and (2) dextrinase, which hydrates the molecule of dextrine to one of maltose. He supposes then that the differences between starch and dextrin are physical, not chemical, and one of his arguments in favor of their

*In true solution each molecule of the dissolved substance lies separate in the dissolving medium, while in colloidal solution the individual molecules are not entirely separated from each other but occur in minute groups—solution aggregates as they are called by Moore and Parker. Substances in true solution will dialyze, but those in colloidal solution with difficulty or not at all according to the size of the aggregates. Substances in colloidal solution therefore are practically in suspension and the only difference between these and insoluble substances is in the size of the suspended particles.

identity is that they both rotate the plane of polarized light to the same degree (a) d+202.0, while maltose rotates (a) d+138.0.

Beijerinck[*1] is of a different opinion, although he also considers that amylase is a mixture of two different enzymes, both of which act upon starch; one forming erythrodextrin and maltose, which he calls erythrodextrinase, and the other forming leukodextrin and maltose, which he calls leukodextrinase. Leukodextrinase can also hydrate erythrodextrin to maltose, but erythrodextrinase has no effect upon leukodextrin. By prolonged action the leukodextrins also are converted into maltose.

Whether amylase is composed of two or more different enzymes or not for the present remains an open question, but it undoubtedly is possessed of two distinct properties; first liquefying starch and then converting it into maltose.

It has been found impossible to obtain amylase or, indeed, any enzyme in a pure state, so that their constitutions are not known, but it is probable that they are of a proteid nature for the most part.

Amylase is secreted by Aspergillus niger, penicillium, glaucum, and several other molds. In European and American breweries the agent made use of for converting malt starch into sugar, as a preliminary to alcoholic fermentation by yeast, is the diastase (amylase) contained in the malt itself.

In China and Japan there are native beers and other alcoholic drinks made from cooked rice, and since any amylase present in the rice must have been destroyed in the cooking, the amylase secreting powers of certain molds are called into action. The active principle used in brewing the Japanese saké is called koji, and consists of a mass of *Aspergillus oryzæ* and yeast with a certain proportion of rice.

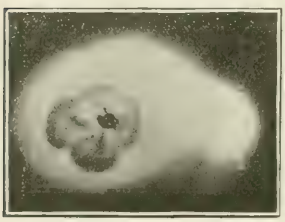


Fig. 1.—(Natural size.) Aspergillus oryzæ on starch agar plate treated with iodin. Transmitted light. White background. A clear ring surrounds the central growth.

On introducing the mixture into thin rice starch paste the aspergillus rapidly attacks

*I have altered Beijerinck's terminology a little to bring into line with that used here.

the starch, converting it into maltose, and the process is continued by the yeast.

The accompanying table is given for comparison :

Drink.	Starch.	Converted into sugar by	Alcoholic fermentation by yeast.
European beer.	Malt.	Amylase secreted by germinating barley.	Saccharomyces cerevisiæ.
Japanese beer (sakê).	Rice.	Amylase secreted by *Aspergillus oryzæ* (mold).	Saccharomyces.(?)
Chinese beer.	Rice.	Amylase secreted by *Amylomyces rouxii* (mold).	Saccharomyces rastorianus.

Bacillus lactis aerogenes and some others found in sewage, water, and soil secrete amylase under favorable conditions, *i. e.*, presence of starch and scarcity of directly assimilable carbohydrates.

To demonstrate the formation of amylase by bacteria an ingenious experiment has been devised by Wysman.

To sugar free broth gelatin .5% or 1% soluble starch is added and after sterilization is allowed to set in a Petri dish, diluted sewage being poured over the surface and poured off again. After 48 hours or so the plate is flooded with diluted Lugol solution, the gelatin quickly turning blue except around those colonies which have formed amylase. Such colonies appear surrounded by a white ring, owing to the conversion of the starch into dextrin and maltose. Sometimes there is an outer ring of a reddish color, showing the presence of erythrodextrin.

Since many bacteria produce alkali, in the presence of which starch does not turn blue with iodin because of the formation of an iodid, it is necessary first to flood the plate with a 4% or 5% solution of HCl, leaving it on for half a minute or so to neutralize the alkalies. If this is not done the alkali producers will show a clear ring around them, not necessarily due to amylase.

Eijckman by means of starch agar plates has shown that anthrax and the spirilla of cholera and Metchnikoff secrete amylase, a clear ring forming around the colonies. *B. coli communis*, typhoid, diphtheria, and some others showed no clear ring or only a trace. He considers the clear ring sufficient evidence and finds it unnecessary to treat with iodin.

I have confirmed Eijckman's observations on starch agar plates and have also demonstrated conversion of starch by cholera and anthrax bacilli in another way.

To sugar free broth ½% of corn starch is added and a number of tubes filled, each with 5 cc. Ten tubes are then inoculated with the organism and each day one taken out and boiled. At the end of ten days all are filtered, 1 cc. of each tested with Fehling's solution and the remaining 3 or 4 cc. after boiling is put into a fermentation tube, together with sufficient sugar free broth to fill it up. The fermentation tubes are then inoculated with *B. coli communis* and the amount of gas formation noted in 48 hours. *B. coli communis* will form gas if sugar is present but not otherwise.

It was found in this way that anthrax began to convert the starch in 48 hours and cholera in three days. Both showed progressive increase in the amount of sugar for seven or eight days, after which there was progressive decrease on the ninth and tenth days, no doubt owing to the utilization of the sugar already formed.

In both cases the amount of starch converted is small, though anthrax shows a somewhat more vigorous action than cholera. Controls were, of course, taken in each instance.

The foregoing experiments, however, only show that certain living bacteria can convert starch into sugar, and this might be taken to be due to vital action, although it would entail the supposition that the starch solution aggregates can be absorbed by the bacterial cell. But if some of the agar on which an old culture of a starch converter has been growing is smeared over the surface of a starch agar plate, to which a little thymol has been added, conversion of the starch can be demonstrated by treatment with iodin after two or three days.

Here there can have been no growth of the organism and the conversion must have been due to a diffusible enzyme previously secreted.

Maltase.—Since amylase only converts starch as far as maltose—a disaccharid—it is necessary for this to be still further hydrated to a monosaccharid, and this is done by means of another enzyme, maltase, which in nature almost always accompanies amylase, converting a molecule of maltose into two of glucose. Maltase has been found by Bourquelot[7] and others in Aspergillus niger, penicillium glaucum, and in most of the yeasts. Little has been done in this direction with bacteria, but it may sometimes be detected in colonies of *B. coli communis* by the following auxanographic method of Beijerinck[1] of great value for the detection of small quantities of enzyme.

Take gelatin 10% in water, KNO 3.1%, KH_2PO_4

.05%, maltose 2%. Boil and then filter off the precipitate of $Ca_3(PO_4)_2$.

In this make a suspension of saccharomyces mycoderma,* and allow to set in a Petri dish. Saccharomyces mycoderma does not attack maltose, produces no maltase in fact, so grows very slightly in this medium. If now streaks of *B. coli communis* are made on the surface maltase is secreted and diffused into the medium, converting the maltose into glucose, which can be assimilated by the saccharomyces mycoderma. This at once begins to grow and colonies of it can be seen springing up in those parts of the gelatin lying below and around the streaks.

The auxanographic experiments with maltase, however, are not always successful, since maltase is very slightly diffusible as compared with amylase. The physiologic reason for this appears to be that starch being insoluble or in colloidal solution, it cannot be taken into the bacterial cell as such, but must first be converted extracellularly. Maltose, on the contrary, can be absorbed by the cell so that the conversion can take place intracellularly, and there is not the same necessity for diffusion into the surrounding medium.

Invertase converts the disaccharid cane sugar into one molecule of glucose and one of fructose.

Invertase occurs in large quantities in yeast and can be easily extracted from this by maceration for some hours in chloroform water and filtering till clear. Its activity can be tested by adding 1 cc. of the extract to 5 cc. or 10 cc. of 10% solution of cane sugar and testing at intervals with Fehling's solution for the presence of glucose.

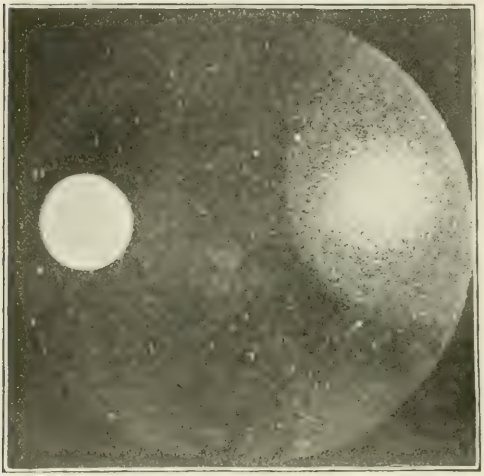
Several molds have been found to secrete invertase, among them Aspergillus niger, from which, however, it is difficult of extraction until actual growth has ceased and the cells begun to lose their vitality.

Duclaux, therefore, grew Aspergillus niger on a large surface of a mineral solution (Raulin's) containing cane sugar for four days, at the end of which time he withdrew the medium and replaced it by sterilized distilled water. The enzyme was then discharged into the water, and after filtration from the mycelium the extract was found to invert cane sugar very readily. Probably this afforded about as pure a solution of an enzyme as has been obtained, although Malfitano finds that a tryptic enzyme is also discharged at the same time.

* To obtain saccharomyces mycoderma, expose a glass of beer for a few days. A film forms on the surface consisting chiefly of mycoderma, which can be isolated by plating out on gelatin made up with brewer's wort.

On repeating this experiment I found the extract contained no tryptic enzyme,* but there was an appreciable amount of amylase. Invertase, however, greatly predominated. On boiling the extract there was no coagulation, showing an absence of ordinary proteid material. Nor did the biuret test reveal the presence of peptones, so that there was probably little dissolved matter in the extract outside of the enzymes.

Although it is generally held that enzymes are of a



Left. Right.

Fig. 2.—Auxanographic plate. 2% cane sugar with suspension of Saccb. mycoderma. Transmitted light black background. Right—Colony secreting invertase colonies of mycoderma all around. Left—Colony not secreting invertase; no visible colonies of mycoderma.

proteid nature it is a fact that solutions can be obtained which afford no proteid reactions and yet contain active enzymes. It has been pointed out by Nencki and Sieber that reactions for proteids have their limitations, the biuret test not being reliable below one in ten thousand, the xanthoproteic below one in a million, and so on,

* My culture of Aspergillus niger does not appear to denote any proteolytic enzyme. (See Table.)

while enzymes can show great activity even when present in infinitesimally small amounts.

Another suggested explanation is, that an enzyme is a proteid molecule plus something else, the additional molecule preventing the proteid from giving the usual reactions.

As is well known to the bacteriologist, some varieties of *B. coli communis* will form acid and gas in cane sugar media (*Coli communis communior*), while others will not (*Coli communis verus*). That the gas-forming cultures secrete invertase may be shown by the experiment with saccharomyces mycoderma already described, substituting cane sugar for maltose. Around streaks of *B. coli communis* cultures, which form gas in cane sugar media, a growth of saccharomyces mycoderma will appear, no such growth being visible around streaks of cultures which do not form gas in cane sugar.

Lactase.—Another disaccharid lactose is converted into glucose and galactose by lactase, first demonstrated by Beijerinck,[2] an enzyme which is secreted by all varieties of *B. coli communis*, but not by typhoid. The paracolons and paratyphoids, which are intermediate between *B. coli communis* and typhoid, form gas in maltose and glucose, but not in cane sugar or lactose media. By means of the saccharomyces mycoderma test it can be shown that these secrete maltase, but not invertase or lactase.

By this method it also appears that Aspergillus niger and Aspergillus oryzæ do not secrete lactase, although both are great producers of amylase and invertase.

A red bacillus isolated from the air, forming a thick, glutinous growth on potato, was found to convert lactose very readily, but had no effect on starch or cane sugar. The bacillus could not be identified with any of those described in the textbooks.

Inulase.—In addition to starch the polysaccharid inulin occurs as a reserve material in most if not all tuberous roots, and can be converted into fructose by inulase, an enzyme first demonstrated by Green in 1886 in the germinating tubers of the artichoke.

Bourquelot[8] showed that Aspergillus niger, that unfailing resource of the enzyme hunter, secretes inulase when grown in a medium containing inulin, and I find this to be the case if inulin is substituted for cane sugar in the Duclaux experiment already described. Aspergillus oryzæ, under these circumstances, secreted no inulase, nor could it be demonstrated with any bacteria by means of inulin auxanographic plates.

Cytase.—Cellulose can be liquefied and hydrated to sugars by means of the enzyme, cytase, which has been shown to be secreted in the aleurone layer of germinating grains, softening the cell walls so that the starch contained in the cells of the endosperm becomes more readily attainable by the amylase secreted by and diffused out from the scutellar epithelium. The accompanying diagram will explain the meaning of this.

Many molds which are parasitic in plants secrete

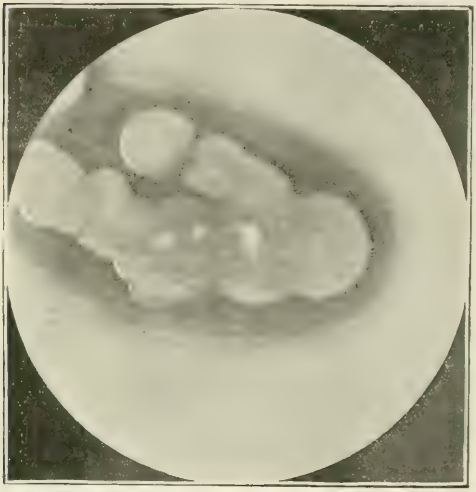


Fig. 3.—Milk agar plate. Anthrax digested around growth. The outer ring of coagulation does not show on the photograph.

cytase, softening and breaking down the cell walls, by which means they are enabled to force their way into the plant tissues on which they feed. De Bary in 1886 first demonstrated the action of cytase in reziza sclerotiorum, a mold parasitic on the roots of carrots and turnips.

Among the bacteria the only ones which secrete cytase in appreciable amounts, and can, therefore, attack cellulose, are certain of the obligate anaerobics, and

among these *Bacillus butyricus* plays a prominent part. This fact is taken advantage of in places where sewage is purified by biologic methods before discharging it into the water-courses.

The purification is effected by running the sewage through tanks and coke beds where bacteria multiply and break down organic substances, partly by means of enzymes and partly by catalysis.

The first tank, the septic tank, is so arranged that the action is strictly anaerobic, and here the cellulose is liquefied and hydrated to such an extent by the enzymes of *B. butyricus* and other anaerobics that in the subsequent coke beds which are conducted under aerobic conditions the process of further reduction to organic compounds can be easily continued by the facultatives and aerobics. If it were not for the preliminary anaerobic action the cellulose contained in paper, rags, etc., would remain indefinitely unchanged and soon choke up the coke beds.

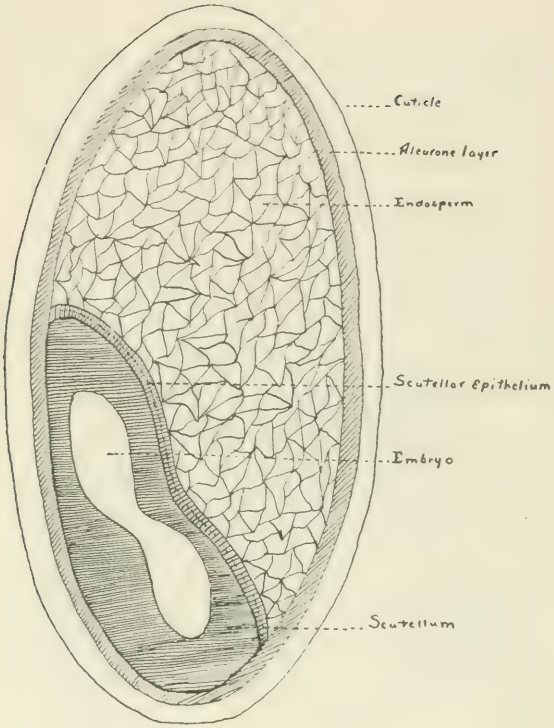
The rotting of flax is due to the action of certain bacteria. In this case, however, it is not the cellulose of the fibers, but the intercellular cement substances, the so-called pectic bodies closely allied to cellulose, which are attacked, being softened and gelatinized by the enzyme pectase, so that the individual fibers of the flax stalks become easily separable and available for the manufacture of linen.

Proteolytic Enzymes.—In the vegetable kingdom and among the lower animals the proteolytic enzymes secreted appear to be more closely allied to trypsin than pepsin, in that they all are able, by prolonged action, to carry the digestion of proteids beyond the peptone stage —purin derivatives, leucin, tyrosin, and other amido bodies being formed.

Certain of the vegetable trypsins have been named after the plants in which they occur, as bromelin from pineapple (Chittenden) and papain from the papaw tree, but as a group the proteolytic enzymes may conveniently be called proteases.

Insoluble and colloidal proteids are probably converted into soluble peptones by hydration, although, as Green points out, there can be no certainty of this since we are entirely ignorant of the constitution of the proteids.

During the purification of sewage the insoluble proteid matter is all reduced to gases, water, and nitrates, and since the action here is purely bacterial it is obvious that the bacteria must secrete proteases in order to start



Grain of Barley

Longitudinal Section [diagrammatic]

Endosperm—Composed of cells containing reserve starch.
Aleurone layer—Where the cytase is chiefly secreted.
Scutellar epithelium—Where the amylase is chiefly secreted.

the process. In artificial purification of sewage the preliminary liquefaction of the insoluble proteids takes place chiefly under anaerobic conditions in the septic tank, and it is known to all bacteriologists that the commoner anaerobics liquefy gelatin and blood-serum.

Of the facultative anaerobics many liquefy gelatin. On taking an old liquefied gelatin culture, killing the bacteria by the addition of a little thymol or chloroform, and pouring into a tube containing fresh gelatin, the latter becomes liquefied more or less rapidly. In this case, since the bacteria have been killed, the liquefaction must be due to a diffusible protease.

That there must be some differences between the proteases secreted by bacteria is shown by the fact that some of those which liquefy gelatin will also liquefy blood-serum, while others will not.

Others again, such as *Streptococcus pyogenes*, do not liquefy either gelatin or serum but can exercise a hemolytic action on red blood-corpuscles.

The ordinary liquefaction of gelatin and serum need not be dilated upon, but demonstration of proteases by means of milk and blood agar plates may be described. Eijckman has worked this out very thoroughly.

Milk Plates.—Ordinary broth agar, 75%, and skim milk, 25%, are separately sterilized, mixed at a temperature of 50° C., poured into Petri dishes and, as soon as set, are streaked with various bacteria. As the latter grow the streak may at first become surrounded by a thickened ring, due to curdling by rennet secreted by the bacteria, and later an inner clear ring appears showing that the casein is being digested. By using litmus milk it can be shown that the process is entirely independent of any acid formation.

Eijckman found that the bacteria which digest casein are precisely those which liquefy gelatin, the nonliquefiers having no effect upon it.

My observations coincide with his, and I find that the agar from old cultures of anthrax and cholera smeared on thymol milk agar plates will produce the same results.

Blood Plates for Hemolysis.—A flask of ordinary agar containing say 100 cc. is melted and cooled to 40° C. To this a little defibrinated blood is added, rapidly mixed, and the agar poured into Petri dishes. The blood-corpuscles remain intact so long as the agar is moist. On the surface streaks are made as with milk plates.

Eijckman found that destruction of the blood-cor-

puscles did not proceed *pari passu* with liquefaction of the gelatin. On smearing a thin sheet of gelatin over the agar, contiguous to the streak, it was observed that anthrax would begin to destroy the blood-corpuscles before the gelatin became liquefied, while with cholera the reverse obtained. From these and other similar observations he concluded that the tryptic and hemolytic enzymes are not identical.

Among those bacteria which do not liquefy gelatin I find that *B. coli communis*, typhoid, and *Streptococcus pyogenes* will destroy human and rabbit blood-corpuscles suspended in agar. The action of the two former seems a little uncertain, but the streptococcus always exerts a hemolytic action, though rather slowly. It is usually 48 hours before the clear ring around the growth appears, but from then on is steadily progressive, attaining a width of 4–5 mm. in four or five days. That some bacteria which do not liquefy gelatin at all can exercise a hemolytic action is another argument in favor of the nonidentity of bacterial hemolysins and ordinary proteases.

Streptococcus pyogenes appears even to have some hemolytic power *in vivo*. Both Bordet and Besredka have observed that in rabbits injected with a virulent culture there is laking of the blood just before death. Other bacteria which can be shown to exert a hemolytic action *in vitro* do not appear to have this power *in vivo*.

The hemolytic principle is diffused out from the bacterial cells into the culture media and in fluid media can be separated from the cells by filtration. In this way staphylolysin (Neisser and Wechsberg), tetanolysin (Ehrlich), typholysin (Levy and Levy), and other lysins have been isolated. Streptolysin, however, does not appear to be so diffusible, and Besredka was only able to separate it by special methods.

Special Methods.—It is a question, however, how far the hemolytic power of bacterial filtrates and cultures is in reality due to ferment action. Levaditi has quite recently shown that extracts of animal tissues quickly made possess very slight or no hemolytic power. This power when it exists is due to a thermolabile (*i. e.*, nonresistant to 60° C.) ferment-like hemolysin. If the process of extraction be prolonged the hemolytic power is greatly increased, but this additional hemolytic principle is thermostable, cannot be destroyed even by boiling, and is due to the presence of amido acids, etc., products of autodigestion, which destroy the blood cells by simple chemical action.

On reading the various reports of experiments on bacterial lysins with this idea in view it strikes one that in many instances their action might be explained in a similar way.

Our blood agar plates then will show us which bacteria can destroy blood cells, but they will not tell us whether the destruction is due to a hemolytic enzyme secreted by the bacteria or to certain of their simple crystallizable by-products.

In all probability further investigation will show that the hemolytic properties of the nonliquefying bacteria are due to the latter, and it is significant that colilysin and typholysin are said to be thermostable, while tetanolysin and staphylolysin are thermolabile (Kayser).

Egg Albumen.—On dissolving 4% or 5% of white of egg in agar cooled to a temperature below that at which the egg albumen coagulates and plating out the medium appears slightly opalescent. If the surface is streaked with various bacteria, some will clear up the agar for several millimeters beyond the growth, and on heating the plates over a flame the egg albumen will coagulate and become quite opaque, except where it has been digested. Fibrin rubbed down fine and suspended in agar is digested by most of the liquefiers.

The accompanying tables have been compiled from my own observations, principally on agar plates:

ENZYMES HYDRATING CARBOHYDRATES.

		Amylase.	Maltase.	Invertase.	Lactase.	Inulase.
1	Anthrax	+	+	—	—	—
2	Cholera	—	+	—	—	—
3	Coli communis	—	+	+	+	—
4	Typhoid	—	+	—	—	—
5	Diphtheria	—	—	—	—	—
6	Staph. pyogenes aureus	—	+	—	—	—
7	Lactis aerogenes	+	+	+	+	+
8	Pyocyaneus	—	—	—	—	—
9	Violaceus	—	—	—	—	—
10	Mycoides	—	—	—	—	—
11	Prodigiosus	—	—	—	—	—
12	Saccharomyces niger	+	+	+	—	—
13	Saccharomyces neoformans	—	+	+	—	—
14	Aspergillus niger	+	+	+	—	—
15	Aspergillus oryzæ	—	—	+	—	—

PROTEOLYTIC ENZYMES DIGESTING

		Milk.		Gelatin.	Serum.	Egg albumen.	Fibrin.	Red blood-corpuscles.
		Coagul.	Digestion.					
1	Anthrax		+	+	—			
2	Cholera	+						+
3	Coli communis	—	—	—	—	—	—	+
4	Staph. pyogenes aureus	+	+	+		—	—	
5	Streptococcus pyogenes	—	—	—	—	—	—	—
6	Pyocyaneus	+	+	+		+	—	+
7	Violaceus	—	—				—	+
8	Mycoides			+	—	+	—	+
9	Prodigiosus		+			+	+	+
10	Aspergillus niger	+	+			—	—	
11	Aspergillus oryzæ	+	+	+	+	+	—	—

Photographs.—1 (natural size). Aspergillus oryzæ on starch agar plate treated with iodin. Transmitted light. White background. A clear ring surrounds the central growth.

2. Auxanographic plate, 2% of cane sugar with suspension of saccharomyces mycoderma. Transmitted light. Black background.

Right: Colony secreting invertase. Colonies of mycoderma all around.

Left: Colony not secreting invertase. No visible colonies of mycoderma.

3. Milk agar plate. Anthrax. Digested around growth. There is not sufficient contrast in milk agar plates to enable a clear photograph to be taken.

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CONSTRUCTION OF THE PROTEID MOLECULE.

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The following is an attempt to set forth in a semipopular way the more recent ideas of the German school, as represented by Hofmeister, of Strasburg, with regard to the construction of the proteid molecule.

To the physiologic chemist, of course, these ideas are well known, but to the general run of the medical profession they are, for the most part, still a sealed book. They were so, at any rate, to me until I recently took a private course of instruction in Hofmeister's laboratory with Dr. Karl Spiro.

By a student with some previous knowledge of theoretic chemistry, coming fresh to the work, I have an impression that the theories can be more clearly explained than by one who has been familiar with the subject from its inception and therefore less able to appreciate the difficulties in the way of a right understanding at the start.

So much by way of apology for the attempt.

The endeavor to solve the mysteries of the proteid molecule by means of ultimate analysis has long been abandoned, but it has gradually come to be recognized that the molecule contains a number of definite nuclei, which can be split off, more or less intact, from the original molecule, and it is by a study of these nuclei and the manner in which they are combined that a solution of the problem may ultimately be attained.

The various nuclei can be obtained by hydrolysis of the original molecule, and hydrolysis can be effected by boiling with diluted acids, or better by digestion with proteolytic enzymes, more particularly trypsin. The point is to apply methods sufficiently drastic to resolve the proteid molecule into its component nuclei without injury to the latter.

For reasons which need not be entered into it is roughly estimated that the average molecular weight of a proteid may amount to about 15,000, and the average molecular weight of the individual nuclei, which can be split off, may be taken at about 120. Very roughly speaking, therefore, there would be about 120 nuclei in

each molecule. The number of different nuclei known at present is about 20, and there may be, and probably are, others which await discovery. It is certain, however, that each molecule may contain more than one nucleus of the same kind, and some of the nuclei in much larger proportions than others. The leucin nucleus, for instance, is found in most proteids to occur much more numerously than any of the other nuclei and the monoamino acids in general more numerously than the diamino acids. Again, certain nuclei which are present in some proteids may be absent in others, but the monoamino acids are always represented.

The principal nuclei known are as follow:

I.—Monoamino Acids.
- *a. Monobasic.*
  1. Glycocoll (aminoacetic acid) $C_2H_5NO_2$
  2. Alanin (aminopropionic acid) $C_3H_7NO_2$
  3. Leucin (aminocaproic acid) $C_6H_{13}NO_2$
- *b. Dibasic.*
  1. Asparaginic acid, $C_4H_7NO_4$
  2. Glutaminic acid, $C_5H_9NO_4$

II.—Diamino Acids.
1. Ornithin (diaminovalerianic acid) $C_5H_{12}N_2O_2$
2. Lysin (diaminocaproic acid) $C_6H_{14}N_2O_2$

III.—Glucosamin, $C_6H_{13}NO_5$

IV.—Cystein (thioaminolactic acid) $C_3H_7NO_2S$

V.—Isocyclic Series.
1. Phenyl alanin, $C_9H_{11}NO_2$
2. Tyrosin (Oxyphenyl alanin) $C_9H_{11}NO_3$

VI.—Heterocyclic Series.
- *a. Indol Group.*
  1. Indol, C_8H_7N
  2. Skatol, C_9H_9N
  3. Tryptophan, $C_{11}H_{12}N_2O$.
- *b. Pyrimidin Group.*
  1. Histidin, $C_6H_9N_3O_2$

Two points may here be noted:

1. The nuclei, with a few unimportant exceptions which need not be considered, contain nitrogen.

2. The NH_2 group in the monoamino acids is always in the alpha position, *i. e.*, attached to the C next to the acid group. In the diamino acids one of the NH_2 groups is always in this position. This fact is of importance for the theory with regard to the construction of the proteid molecule.

In considering the means by which the nuclei may be combined certain conditions must be complied with.

1. The nuclei must be linked together in such a way that they are capable of being separated by hydrolysis, since it is by hydrolysis that they are obtained.

2. They must be linked in such a way that the resulting molecule does not acquire very pronounced acid or basic characters. The proteid molecule, like the amino acids of which it is principally composed, has a Janus face. It is slightly acid on account of one, or not more than a few, COOH groups, and at the same time slightly basic on account of one, or not more than a few, NH_2 groups. The arrangement must be such that neither of these groups predominates over the other.

There are three principal ways in which the nuclei can be linked together:

(A) $-C-C-$ (B) $-C-O-C-$ (C) $-C-NH-C-$

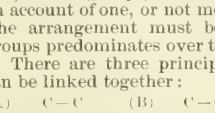
$-C-O-N-$ may be thrown out at once, since $R-COOH + NH_2-R$ would always produce $R-\underset{|}{CO}-NH-R + H_2O$

so that O can never hold an intermediate position between C and N.

A. $\equiv C-C\equiv$. Two possible combinations.

EXAMPLES.

I.

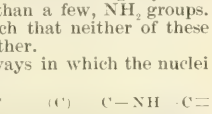


Leucin — Asparaginic acid

POSSIBLE.

But implies numerous ketone groups and no enzymes are known which can split a ketone, so the combination is improbable. Although in this case there would be an excess of NH_2 groups their basic character would be neutralized.

II.



Leucin — Asparaginic acid

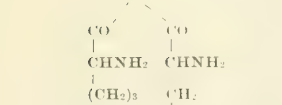
IMPOSSIBLE.

No hydrolysis is possible, so it does not comply with the first condition.

B. $\equiv C-O-C\equiv$. Two possible combinations.

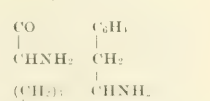
EXAMPLES.

I.



Leucin — Asparaginic acid anhydrid

II.



Leucin — Tyrosin Ester

IMPOSSIBLE.

A combination which would be unstable and lead to strongly basic characters, so does not comply with the second condition.

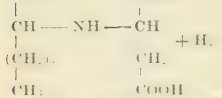
CONCEIVABLE.

But could only occur rarely, as except for tyrosin there are no alcohol groups in the nuclei. If the tyrosin were combined in this way the proteid molecule would not give Millon's reaction which is due to a free OH group on an isocyclic ring.

C. $\begin{matrix} C-NH-C \\ \| \quad\quad \| \\ C \quad\quad C \end{matrix}$. Two possible combinations:

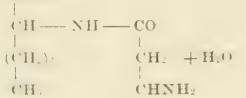
EXAMPLES.

I.



Leucin Asparaginic acid

II.



Leucin Asparaginic acid

IMPOSSIBLE.

No hydrolysis possible and would lead to strongly acid characters, so complies with neither condition.

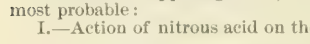
THE BEST.

Stands the two conditions and is probable for other reasons.

Reasons for supposing the C,II combination to be the most probable:

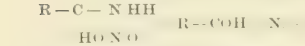
I.—Action of nitrous acid on the proteid molecule

a. If NH_2 groups are treated with HNO_2 the result is:



There is a release of nitrogen.

b. If NH groups are treated with HNO_2 the result is:



There is no release of nitrogen, but formation of nitroso combinations.

On treating proteids with HNO_2 there is very little release of nitrogen, implying a scarcity of NH_2 groups.

Paal found that on treating glutin peptone with HNO_2 nitrosamins were formed, implying the presence of NH groups.

II.—*The Biuret Reaction.* For the biuret reaction certain special dispositions of the C groups with a free NH_2 group are required, and these are afforded by the C,II combination. The A,I combination would also give them, but in this case there would be such a large number of free NH_2 groups that there would be as many combinations in the original molecule to afford the reaction as in the intermediate albumoses and peptones. The reaction, however, goes on increasing in intensity so long as the latter are being formed, and this is exactly what would happen with the $\equiv C—NH—C\equiv$ combination, since there would be a constant accession of free NH_2 groups as hydrolysis progressed.

On treating proteid with HNO_2 the molecules are hardly affected as regards their total N content, but lose their power of affording the biuret reaction. This means that the NH_2 groups which are destroyed by the HNO_2 must be comparatively few in number.

III.—*Amino Acids have a Tendency toward Condensation in this Manner.* Curtius synthesized a base which gave the biuret reaction and could be hydrolysed by trypsin. The base was found to be an ethyl ester, containing 7 glycocoll nuclei joined by means of their COOH and NH_2 groups.

$$H_2N-(CH_2-NH-CO)_6CH_2.CO.O.C_2H_5$$

The bracketed portion of this may be partially detailed.

$$\begin{array}{ccccccc} & & HO\ H & & HO\ H & & HO\ H \\ \cdot NH-CH_2.CO- & NH- & CH_2.CO- & NH- & CH_2.CO- & NH- & CH_2.CO- & NH- \\ & | & & | & & | & \\ & H & & H & & H & \end{array}$$

The dotted lines show where synthesis occurred and where splitting would occur on hydrolysis.

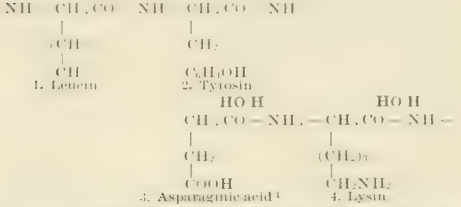
IV.—*Similar Syntheses Occur "in Vivo."* On feeding benzoic acid it combines with glycocoll and appears in the urine as hippuric acid.

$$C_6H_5.COOH + CH_2NH_2.COOH$$
$$C_6H_5.CO-NH.-CH_2.COOH + H_2O$$

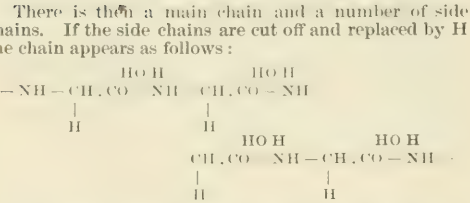
In this case the combination occurs between the COOH and NH_2 groups.

It appears then more than probable that linkage of the various nuclei occurs by means of the N_2 and bearing in mind that in the amino acid nuclei the NH_2 group is always in the alpha position, we can construct a

portion of a proteid molecule as follows, the dotted lines showing where hydrolysis can occur:



There is then a main chain and a number of side chains. If the side chains are cut off and replaced by H the chain appears as follows:



or the detailed portion of the Curtius base over again, and it becomes obvious that the whole thing may be regarded as a substituted glycocoll chain; the substituting side chains being in the case in point: 1. Butane. 2. Methyl paraoxybenzene. 3. Acetic acid. 4. Butylamin.

Surmising then that the molecule consists, for the most part at any rate, of a main glycocoll chain with a series of free swinging chains, none of which by the way is very long, it may be asked in what way is this of interest to the physiologist. There are certain inferences which can be drawn from the theory, which help to explain the processes of metabolism in the body.

If proteids are treated with permanganate there is oxidation but no hydrolysis. In this case certain peroxyprotacids arise, complicated acids which give the biuret test. Such acids are very easily split up and afford ammonia, some glycocoll, but above all oxalic

[1] It will be understood that the tyrosin and asparaginic acid are linked together.

acid. There is here, according to the theory, no hydrolysis of the main chain, but the side chains are gradually whittled away by oxidation.

I.—

$$-NH-CH.CO-NH-CH.CO-NH-CH.CO-NH-$$
$$\quad\quad|\quad\quad\quad\quad|\quad\quad\quad\quad|$$
$$\quad C_4H_9 \quad\quad\quad C_7H_7O \quad\quad\quad C_4H_8NH_2$$
$$\quad \text{Leucin} \quad\quad\quad \text{Tyrosin} \quad\quad\quad \text{Lysin.}$$

II. By oxidation we get :

$$NH-CH.CO-NH-CH.CO-NH-CH.CO-NH-$$
$$\quad\quad|\quad\quad\quad\quad|\quad\quad\quad\quad|$$
$$\quad COOH \quad\quad\quad COOH \quad\quad\quad COOH$$

III. The CH groups of the main chain are next oxidized and CO_2 is split off so the chain appears as :

$$-NH.CO.CO-NH \quad CO.CO \quad NH-CO.CO-NH-$$

IV. Such a combination would be unstable and would split up by hydrolysis of itself without any stimulus in the shape of a ferment and become

$$(CONH_2.COOH)n = \text{amino oxalic acid.}$$

V. Amino oxalic acid readily hydrolyses to oxalic acid.

$$CONH_2.COOH + H_2O = COOH.COOH + NH_3$$

Such parts of the main chain as had not undergone the final oxidation would appear as glycocoll.

This scheme of grouping, therefore, explains the products of pure hydrolysis on the one hand and of pure oxidation by permanganate on the other.

In the body, hydrolysis and oxidation go on hand in hand, so that the main chain can be hydrolysed and the side chains oxidized to suit the needs of the organism, and glycocoll might be one of the chief products. It seems curious that neither glycocoll nor any other product intermediate between proteid and urea is ever found in fresh tissue or organs, but such products would, in any event, be present in very small amounts, and presumably when once a proteid molecule begins to break up, its remains are very speedily disposed of. That glycocoll is available when needed is shown by its attaching itself to benzoic acid to form hippuric acid.

Hofmeister shows how glycocoll may be made available. Taking Table II of the oxidation series we find :

$$\quad\quad HO\ H \quad\quad\quad HO\ H \quad\quad\quad HO\ H$$
$$-NH-CH.CO-NH-CH.CO-NH-CH.CO-NH-$$
$$\quad\quad|\quad\quad\quad\quad|\quad\quad\quad\quad|$$
$$\quad COOH \quad\quad\quad COOH \quad\quad\quad COOH$$

If hydrolysis occurs at this point we have a number of amino malonic acid nuclei ($COOH.CHNH_2.COOH$), and amino malonic acid very easily, by slight warming even, splits into glycocoll and CO_2.

$$COOH.CHNH_2.COOH = COOH.CH_2NH_2 + CO_2$$

Glycocoll, according to Hofmeister, can readily be oxidized to urea, NH_2 being annexed at the same time. It is well known that NH_3 is being constantly liberated in certain organs, *e. g.*, the liver, although it does not leave the body as such, but as urea. Glycocoll and NH_3 are, therefore, constantly available, and what happens may be represented by the formula:

$$\underset{\text{Glycocoll}}{CH_2NH_2.COOH} + NH_3 + 3O = \underset{\text{Urea.}}{CO(NH_2)_2} + CO_2 + 2H_2O$$

Summarizing then we have as products:

In pure hydrolysis, aminoacids.
In pure oxidation, oxalic acid.
In mixed hydrolysis and oxidation, glycocoll and finally urea.

It is not of course intended to imply that all combinations between the nuclei occur in this manner, but only, in all probability, the large majority of them. At least one exception is known, that of arginin which is a combination of guanidin and ornithin.

$$\underset{\text{Guanidin}}{HN=C(NH_2)_2} + \underset{\text{Ornithin.}}{CH_2NH_2.CH_2.CH_2.CHNH_2.COOH} =$$

$$\underset{\text{Arginin.}}{HN=C(NH_2)-NH.CH_2.CH_2.CH_2.CHNH_2.COOH} + NH_3$$

Here the synthesis occurs without loss of H_2O and consequently arginin is not split up by hydrolytic action. On hydrolysis ornithin and guanidin are not found as such among the final products but as arginin. It will be noticed however that this combination does not affect the glycocoll end of the molecule which could attach itself to the main chain in the ordinary way, the only difference being that in this case we have a binary compound swinging from the main chain instead of a single nucleus.

Such in brief is the theory; a theory which, however imperfect and capable of modification, does certainly appear to throw some light upon the construction of the proteid molecules and the way in which they may be utilized by the organism.

A CASE OF SEPTICEMIC GLANDERS IN THE HUMAN SUBJECT.[1]

WARREN COLEMAN, M.D., and JAMES EWING, M.D.

(*Cornell University Medical College, New York City.*)

The comparative rarity of the occurrence, or at least of the recognition of glanders in the human subject, and the somewhat remarkable features of this case, seem to warrant the detailed report of the study of a case of glanders examined by the writers in September, 1901. This case is the fourth, according to the records of the Health Department, that has occurred in New York City since 1888, viz., one in 1888, one in 1893, and one in 1899; but Park failed to obtain the glanders bacillus from the material of the third case, and believes that it was really not glanders.

CLINICAL HISTORY.

John E. D., aged 36 years, hostler, and occasional "veterinary surgeon" by occupation, was admitted to Bellevue Hospital, Aug. 24, 1901. Three weeks previously the patient had been bitten by a horse, said to have "distemper," on the third finger of the left hand. The wound suppurated, was treated by a physician, and healed after ten days. The patient had no constitutional symptoms until the wound had healed, or about ten days before admission. So far as could be elicited from him or his friends, there had been no involvement of the lymph nodes of the arm.

The illness began with headache, disinclination to exertion, vague muscular pains in the back and legs, loss of appetite, and diarrhea. There was neither chill, epistaxis, nor cough. The patient grew progressively weaker, and sought admission to the hospital.

August 24th.— Physical Examination by Dr. G. C. Buck.— The patient is well nourished and muscular. The face is flushed and apathetic; the eyes are suffused and dull. The tongue is coated over the centre, red at the tip and edges, and markedly tremulous. The pulse is 88, regular, of good force, and compressible; the temperature is 104.6°.

The heart is normal in size, the sounds are of fair quality and unaccompanied by murmurs. There is no abdominal distention or tenderness. A few rose-colored spots, somewhat similar to those of typhoid fever, are

[1] Received for publication March 5, 1903.

present over the upper part of the abdomen. The liver is normal in size. The spleen is not palpable, but the area of splenic dullness is slightly enlarged. The wound of the finger is completely healed.

August 25th.— The patient is more apathetic, and at times slightly delirious. A tender fluctuating swelling has developed in the middle outer anterior aspect of the left leg. The overlying skin is red. The "rose spots" on the abdomen are more numerous, and some are distinctly raised above the surface.

August 26th.— The patient is very talkative, and at times actively delirious. The swelling on the left leg has grown larger. About 6 oz. of thick, grayish, blood-streaked pus is discharged after incision. Some of the "rose spots" on the abdomen have become distinctly papular, and a few small papules have appeared on the forehead, face, chest, and back. A swelling which does not fluctuate has developed in the middle third of the left forearm. The leucocyte count is 13,000.

August 27th.— The general condition of the patient is unchanged. There is very little discharge from the abscess of the left leg. The swelling in the left forearm fluctuates. About 4 oz. of pus is evacuated by incision. Microscopic examination of the pus shows cocci only. A few of the papules previously noted have become pustular. New papules have appeared in the same regions. About this time the patient began to emaciate rapidly.

August 28th.— The patient is delirious most of the time and is decidedly weaker. There is almost no discharge from the abscesses. Several new abscesses are present to-day — one in the right forearm, fluctuates, overlying skin normal; one in the outer part of left thigh, three inches above the knee-joint, does not fluctuate; one in the outer middle third of the right leg fluctuates and is red and tender. The patient has developed a short, hacking, non-productive cough. Widal's reaction is negative.

August 29th.— The delirium continues. The patient is oblivious to all that happens around him. Some of the pustules have been ruptured by the nurse in washing the patient, and are excavated. Some appear umbilicated.

August 30th. The patient's general condition is much worse. Numerous subcrepitant and mucous räles are heard over both lungs. The breath sounds are roughened (the patient talks constantly, preventing a satisfactory examination). The papules and pustules are much more numerous. About 3 oz. of thin grayish pus is obtained from the abscess of the right forearm. The leucocyte count is again 13,000.

August 31st.— The patient is very weak, and has a low, muttering delirium. The eruption is more abundant. Smear preparations made from the pustules contained cocci and a few bacilli possessing the morphological characters of *B. mallei*. Cultures were made from several pustules and an abscess, and 5 c.c. of blood was taken from a vein at the bend of the elbow for bacteriological examination.

September 1st.— Death occurred at 7.45 A.M.

REMARKS ON THE CLINICAL HISTORY.

The abscesses which developed in the extremities showed a remarkably symmetrical distribution. All of them were deeply seated, and for the most part without signs of inflammation in the overlying skin. The pus flowed readily from the abscess cavities, was of a dirty grayish color, and often blood-streaked. It contained very few organisms, and at the first examination only cocci were found. The abscess cavities did not have a pyogenic membrane. The pus formed within the muscles, but ruptured through them and spread along their sheaths for variable distances. The abscess in the lower part of the left thigh had burrowed nearly into the knee-joint. Practically no reaction took place in the incision wounds. The abscess cavities remained nearly dry.

The eruption appeared about the eighth or ninth day of the disease as a few macules scattered over the anterior abdominal wall. They resembled in some respects the "rose spots" of typhoid fever. It was demonstrated that some of the early macules disappeared without reaching the papular stage, as several, identified by surrounding them with an indelible pencilmark, had left no trace at subsequent examination. By far the majority of them developed into papules which subsequently became pustular. The eruption came out slowly at first, then with great suddenness, almost over night numerous papules and pustules appeared on the forehead, face, chest, and upper part of the abdomen. While the eruption was chiefly confined to these situations, scattered lesions were found over the scalp, arms, and back. During the early part of the disease, the change from macule to papule and pustule proceeded slowly, occupying two or three days, but later the transition was measured by hours. The individual lesions were acuminate papules or pustules situated on a hyperemic base, which became anemic after death. Woodhead, in Allbutt's system, states that the base is sometimes anemic during life.

Many of the lesions ruptured spontaneously or from traumatism and were then umbilicated, but during life and after

death many of the unruptured pustules were distinctly umbilicated. The majority of the small lesions, however, were acuminate. The individual lesions resembled the pustules of variola, but were less numerous, most of them were acuminate, and their comparative scarcity on the face left no ground for confusing the disease with variola. Most of the lesions were discrete, but in several localities there was more diffuse involvement of the derma where the papules and pustules were conglomerate, and areas from two to four centimeters in diameter were indurated, swollen, and reddened. Some of these would undoubtedly have ruptured, leaving deep ragged ulcers, had the patient lived longer. (Fig. 2.) The nasal mucous membrane gave no evidence of involvement during life.

The cough was due to lesions of the trachea, bronchi, and lungs, which will be fully described in the pathological report. The rate of respiration became accelerated during the last three days of the illness, reaching forty per minute. None of the accessible lymph nodes were swollen.

The range of temperature was high. Only once did it fall below 103° F. to 102.8° F., and the highest point reached was 105.6° F.

Until within two days of death the pulse rate was remarkably low, when one considers the temperature curve, varying from seventy-eight to one hundred per minute.

The urine examined the day after admission was pale, acid, of 1,005 specific gravity, and contained neither albumin nor sugar. The microscopic examination was negative. The urine was not examined again.

There was slight diarrhea during the course of the disease.

The treatment was confined entirely to supporting and hydrotherapeutic measures. As the attempts to reduce the patient's temperature by cold baths were futile, and as they seemed to distress him, they were discontinued.

The diagnosis of acute glanders was not made without difficulty. The high temperature, the disproportionately low pulse rate, the spots, resembling as they did at first the

rose spots of typhoid, the tremulous coated tongue, together with the season of the year, naturally suggested typhoid fever. But the day after admission, when the abscess of the leg had appeared and some of the "rose spots" had changed to papules, the idea of typhoid fever had to be abandoned. The pus from an abscess was examined for whatever organisms might be present and only cocci found. As fresh abscesses appeared and as the eruption became pustular, the history of the horse-bite took on greater significance, and the pus was searched anew for bacilli.

At this second examination a few bacilli were found presenting the morphological characters of B. mallei, and pending the result of the bacteriological examination of the blood a provisional diagnosis of acute glanders was made. The post-mortem findings and the cultures from the blood subsequently confirmed this diagnosis.

The incubation period. — Woodhead states that the incubation period after inoculation is very short, twenty-four hours, unless the inoculation takes place through an old wound or the infection is cryptogenic, when the period may be prolonged to one or two weeks or more. Moreover, after inoculation involvement of lymph nodes is prominent.

If these rules apply to the present case, infection could not have occurred when the wound of the finger was received. It may have occurred through this wound during its healing, but this appears unlikely as the wound was treated aseptically and healed after some suppuration. It is still less probable that the infection was of cryptogenic origin, and considerable doubt has been thrown upon the genuineness of some reported infections of this type. In view of the clear history of a bite from a sick horse said to have "distemper," and of a pyogenic infection of this wound, it would seem to us most reasonable to conclude that the glanders infection occurred when the finger was bitten or shortly thereafter, and that dissemination of the bacilli occurred much more slowly than is indicated in the rules laid down by Woodhead. In Duval's case the outbreak of the disease in acute form occurred fifteen days after a wound of the finger.

PATHOLOGICAL REPORT.

Autopsy thirty hours after death.

The *skin* exhibited several hundred papules, macules, and pustules, irregularly distributed over the body, varying in size from a pin head to 1 cm. in diameter. They were generally acuminate, sometimes slightly umbilicated, while the largest lesions were ulcerated and excavated. Many of them resembled variolous pustules, but were less distinctly purulent and not markedly umbilicated. They were surrounded by a peculiar anemic halo ½ cm. in diameter. In some areas the lesions were confluent.

The *skeletal muscles* were the seat of about a score of abscesses ½ to 3 cm. in diameter, containing thick blood-stained pus.

The *heart* was normal.

Both *lungs* exhibited numerous foci of consolidation, resembling small and large tubercles, single or more often conglomerate. These nodules projected slightly on section and on the pleural surface. They were quite firm, and were surrounded by areas of hemorrhage. Numerous larger consolidated foci were present in both lungs. The upper part of the left lung was slightly consolidated and of light yellowish-gray color. Here the pleura was coated with fibrin. The bronchial nodes were very hyperemic. Two of them were necrotic and softened, and one of these had ruptured into the left bronchus, producing a large ragged ulcer of the mucosa. The pneumonic area had apparently resulted from the aspiration of the contents of this node. The bronchial mucosa was very hyperemic, and presented a few prominent papules and pustules.

The *lymph nodes* of the left axilla, groin, and arm were not palpable, and were not dissected out; and no other lymph nodes were found to contain specific lesions.

The *spleen* was the seat of well-marked acute hyperplastic splenitis.

Liver and kidneys showed acute degeneration.

Cranium. Over the right side of the occipital protuberance was a subperiosteal collection of thick pus, and between the dura and skull a similar collection. The meninges were hyperemic and edematous, but the brain was normal.

The gastro-intestinal tract showed slight catarrhal inflammation. Peyer's patches were atrophic.

Bone marrow cellular and hyperemic.

Testicles and bladder normal.

There is a healed wound with irregular hyperemic scar on the tip of the left third finger.

Anatomical Diagnosis. — Pustular eruption of the skin and bronchial mucosa. Multiple intra-muscular abscesses. Necrotic bronchial lymphadenitis; rupture into the bronchus. Multiple granulomata and diffuse pneumonia of the lungs. Glanders.

MICROSCOPICAL EXAMINATION.

The pulmonary lesion. Throughout the lungs there is marked congestion, edema, and considerable catarrhal desquamation of epithelium.

The glanders nodules consist of a series of vesicles filled with exudate and surrounded usually by a zone of hemorrhage. The exudate consists of about equal proportions of mononuclear and polynuclear leucocytes, of exfoliated epithelium, and a considerable admixture of red cells. Fibrin is abundant in many places, and some vessels are filled exclusively with fibrin masses, but in the more cellular portions fibrin is absent. The exuded cells exhibit various degenerative changes, including karyorhexis, karyolysis, hydrops of nuclei and cytoplasm, and fatty degeneration. No collections of fat, such as described by some writers, could be detected.

A great many fusion giant cells are present, of many sizes, and exhibiting a variety of degenerative nuclear changes. The appearance of this exudate and especially of the giant cells is very characteristic. (See Fig. 2.)

It shows some resemblance to acute necrotic tuberculosis, but the giant cells are very different from those of tuberculosis, the necrosis is nowhere complete, and karyorhexis is much more prominent. Any tendency of the glanders bacillus to induce a productive lesion is entirely obscured by the intense exudative process.

The bronchi are the seat of an intense purulent catarrh.

The cutaneous lesion. The inflammatory foci are located in the derma close beneath the epithelium, or very often deeper near the subcutaneous fat. The larger lesions are conglomerate nodules composed of several small foci in which the exudate has gathered in a small cavity, marked off by the distended connective tissue fibres. The exuded cells appear to be principally mononuclear leucocytes, with fewer polynuclear cells, but the necrotic process is too far advanced to allow accurate identification of the cells.

There are some of the same fusion giant cells described in

the lung. Karyorhexis is very advanced, very few cells are left with intact nuclei, and the whole mass of exudate exhibits very numerous pyknotic or hydropic nuclear fragments yielding a very characteristic appearance.

The necrotic area is surrounded by a zone of vessels showing great abundance of fibrin.

In the exudate the red cells have entirely disappeared. In the zone of vessels with excess of fibrin hemolysis has occurred, leaving only shadows, while on the outskirts of the lesion many small vessels exhibit thrombi of agglutinated red cells of homogeneous lustre and often forming the central points of fibrinous coagula.

The anemic zone surrounding the pustule appears to be referable, at least in part, to the numerous small dilatations of the sub-epithelial lymphatics which have raised the epithelium to such an extent as to readily explain its whitening.

The intra-muscular abscesses. (Fig. 1.) The process in the muscles is very similar to that in the lung and skin. The necrotic exudate is identical in appearance with that of the skin lesions. The advance of the destructive process is everywhere marked by a narrow zone of hemorrhage and edema. Later comes the cellular exudate of the usual type. The muscle fibres undergo a series of changes ending in their complete destruction. These consist in swelling, obliteration of Cohnheim's fields, invasion by leucocytes, vacuolation, and fragmentation. Many fibres dissolve without invasion by leucocytes, leaving a faint unstained network as if from complete hydrolysis. Half dissolved or completely dissolved fibres are sometimes seen a considerable distance from the necrotic zone. No trace of reparative proliferation of fixed cells could be discovered. The edges of the necrotic foci are often sharply marked off from very slightly altered muscle fibres. The red cells at a distance from the abscesses often show peculiar agglomeration, but no distinct agglutination, although some solution of Hb has occurred and many crystals of hematoidin are everywhere present.

The marrow. The chief characteristic of the bone marrow, as found in the ribs and vertebræ, is an extreme

congestion of the vessels. The sinuses are gorged with blood, many are distended much beyond their natural limits and some are ruptured, giving rise to minute extravasations. The pulp cords are, as a rule, not more cellular than normal, but in certain scanty and narrow foci there is some increase in large neutrophile myelocytes. The lymphocytes also appear in normal numbers, and islands of nucleated red cells are numerous. A marked excess of fibrin is found in many small vessels — with this exception, the appearances are identical with those seen in the marrow of typhoid fever. No distinct hematomata as described by Ferrari and Guarnieri were found, but the bones of the extremities were not examined.

BACTERIOLOGICAL REPORT.

(*a.*) Circulating blood. On August 31, twenty-six hours before death, five cubic centimeters of blood were aspirated from the median basilic vein, and kept on ice until September second. The blood was then divided between two tubes of ordinary broth. After twenty-four hours in the thermostat no growth was visible, but on the morning of September fourth (forty-eight hours) both tubes exhibited a rather profuse growth, resembling that of streptococcus pyogenes, and consisting of opaque, whitish flocculi which adhered to the sides of the tube and to each other, leaving the fluid clear. The growth consisted of micro-organisms similar to Bacillus mallei and in pure culture. Cultures on blood serum of the blood post-mortem gave colonies of the glanders bacillus and of various unidentified cocci.

(*b.*) Pustules. On August twenty-ninth smears from the pustules contained a few staphylococci and a few bacilli resembling B. mallei. Post-mortem, from a pustule of the skin previously pencilled off with five per cent formalin and sterile water, a growth was obtained of the glanders bacillus and of a large unidentified coccus, while the smears contained only bacilli.

(*c.*) Intra-muscular abscesses. The smears of the pus contained bacilli and cocci, and cultures gave a growth of B. mallei and staphylococcus aureus.

(*d.*) Lung. The sputum examined during life was found to contain many bacilli resembling B. mallei.

Smears of the lung contained cocci, bacilli resembling B. mallei, and a large filiform bacillus. Cultures gave Bacillus mallei in profuse growth, and a large filiform bacillus. The earliest identification of B. mallei was obtained from a culture of the lung which gave a diffuse brownish growth on potato, at first pure, later overgrown by the large filiform bacillus. This growth was obtained in less than twenty-four hours. In some serum tubes inoculated from the lung, Staphylococcus pyogenes aureus grew.

The bacteriological examination therefore gave a pure culture of B. mallei from the circulating blood during life and the morphological demonstration of this micro-organism in the sputum and pus from the abscesses; while after death the same bacillus was obtained from the same sources, but combined always with Staphylococcus pyogenes aureus, and in the lung with a large filiform bacillus. As the staphylococcus was not found in the circulating blood during life, but only in the post-mortem material, and as this micro-organism is so frequently present in the lung post-mortem, we are not inclined to attach much importance to its demonstration in any of the localities except in the abscesses where there seems to have been an active mixed infection.

In a case reported by Duval and others the glanders bacillus was obtained in pure but scanty culture from the circulating blood during life, but the writers can find no other reports of the bacteriological examination of the circulating blood.

Distribution of B. mallei in stained sections. Although the glanders bacillus is demonstrated with some difficulty in sections of tissue stained by ordinary methods, no great trouble was experienced in identifying this micro-organism in thin paraffin sections of the formalin-hardened tissues stained twenty-four hours in saturated watery solution of Ehrlich's rectified methylene blue.

In such preparations the bacillus could be identified especially by the pale staining spots which give it a somewhat

serrated appearance. The bacillus was present in large numbers in all the lesions. Most of them were contained in medium-sized and large mononuclear cells and in the giant cells, in all of which cells they lay singly or in groups, often within distinct vacuoles. Some well-preserved cells contained large numbers of bacilli which distended the cell body. In the outskirts of the nodules where the cells were well preserved, nearly all the bacilli were intra-cellular, but in the necrosing centres many were found extra-cellular.

In sections of the skin the bacilli were present in moderate numbers, and none but those resembling B. mallei could be detected. In the abscesses and in the testicle staphylococci also were detected, while in the lung the glanders bacillus was considerably overgrown with cocci and filiform bacilli which latter were usually extra-cellular.

Some alteration in the intra-vitam numbers and position of the micro-organisms in the tissues must be granted to have occurred after death.

Experimental glanders in the guinea-pig. The usual positive results of inoculation of a male guinea-pig were obtained.

This pig was inoculated intraperitoneally with an emulsion of lung tissue on September third. It did not appear to be much affected till September eighth, when it rather suddenly collaped and died September ninth. The testicles were painful and partially fixed by the end of the third day, and later the scrotum became moderately swollen and edematous.

The autopsy showed a few small collections of caseous material in the skin and muscles at the point of puncture, while on the peritoneal surface adjoining were many fine granules. The visceral peritoneum was shiny. Ascites absent. There was one large pustule projecting prominently from the peritoneal surface of the small intestine. The omentum was shrivelled up into a globular mass one centimeter in diameter and exhibiting several small glanders nodules. Kidneys negative. Lungs, a good many minute grayish foci could be distinguished on section. The Malpighian bodies of the spleen were over-prominent. The liver showed numerous minute whitish foci on section.

The testicles were much swollen, together measuring four centimeters across. They were adherent to the scrotum, the cavity of the tunica vaginalis here being obliterated by a fusion of its layers from the inflammatory process. The lesions involved the entire surface of both testicles and epididymi and extended one centimeter up the cord. Sometimes only one testicle is involved. (Frothingham.) The neighboring parietal peritoneum of the abdominal cavity exhibited a score of fine superficial glanders nodules.

Microscopically the lesions of the testicles were found to have originated on the peritoneal surfaces which were fused together, while the process had spread through the muscular tissue of the scrotum and very often into the substance of the testicle.

In the epididymus the lesions were more extensive and had reached a suppurative grade with the production of small abscesses. Elsewhere the lesions were of the more typical type described in the human tissues. In the lungs and liver the whitish foci proved to be minute glanders nodules. In the liver there were innumerable nodules, the smallest involving but one or two cords of cells in which the liver cells and a few leucocytes were necrotic, nuclei subdivided and pyknotic. No mitosis could be detected. These nodules were identical in appearance with those described by Wright. Many lay alongside the small hepatic veins. Glanders bacilli in intra-cellular groups and fewer extra-cellular individuals were abundant. In the lungs there were the same evidences of focal necrosis about groups of bacilli, as well as some larger nodules.

Cultures of the heart's blood remained sterile.

Cultures from the testicles gave on broth Staphylococcus pyogenes aureus, on blood serum and potato B. mallei and Staphylococcus pyogenes aureus. The growth on potato was of typical brownish color and not whitish as seen with Kutscher's bacillus. Later a greenish shimmer appeared on the edges of the growth. The suppurative character of the lesions about the epididymus may perhaps be referred to the presence of the Staphylococcus pyogenes aureus.

EPICRITICAL.

In the literature on glanders there has always been diversity of statement and opinion regarding many features and even regarding the essential nature of the process. The observations in the present case, although not sufficiently extensive to warrant positive conclusions concerning all forms of the disease, yet have some bearing on the discussion of the disease in its peracute type.

I. Morphology and the distribution of the bacilli in the tissues. An extreme variety in the morphology of Bacillus mallei in cultures has been demonstrated by many observers, especially by Valerio. These described forms include, beside the usual short rods with pale staining segments, enormously elongated threads, chains, polar swelling, large uniformly swollen rods, extremely minute rods, and branching forms. Most of these irregular types appear to be limited to old cultures, but some have been described in tissues. These characters have necessarily led to the classification of Bacillus mallei as a streptothrix. These characters are perhaps most apparent in the tendency of the micro-organism to form large zoöglea masses, "*drusen*," in the tissues, similar to the colonies of actinomyces, as described by Loeffler, Mayer, and others.

In the present case most of the streptothrical characters were entirely absent. In the sputum, pus, and in the early cultures, practically the only form seen was a short bacillus, with pale stained areas, sometimes slightly swollen at one or both ends, sometimes moderately elongated. Here there was not much greater variation in morphology than is to be seen in the tubercle bacillus in the ordinary case of acute tuberculosis.

The early growth on potato was especially uniform in morphology, the bacillus appearing as a very short rod, usually single. Later these forms were replaced by longer ones and some threads, but the great variety of types described by Valerio did not appear. On blood serum the usual forms were striated rods, polar swelling, forms

resembling chain cocci, and some resembling much swollen cocci. No attempt was made to follow the changes in morphology further, as our cultures seemed to show a tendency to remain uniform.

In the tissues the bacilli maintained the same general uniformity, the striated rod being the predominant form. In the skin and abscesses, however, elongated rods or threads or chains were sometimes encountered and many swollen forms resembling cocci were seen.

Although often seen in densely packed groups within epithelioid and giant cells, the micro-organism exhibited no tendency whatever to form large colonies as described by Mayer in experimental glanders, and occurring as the rule, in actinomycosis.

All of these observations accord with the conclusion of Conradi, that pleomorphism is rather strictly limited to the saprophytic existence of the bacillus, and that when growing as a parasite in the animal organism its forms are limited, many phases of development are omitted, and chain and branching forms never appear.

II. The nature of the glanders process. There are at present three main contentions regarding the essential nature of the inflammatory process excited by the bacillus mallei.

(*a.*) The essential feature of the glanders process is a proliferation of fixed tissue cells, with the formation of epithelioid cells and followed by necrosis and exudation. This view is held by Baumgarten, Loeffler, Lubarsch, Strube, and others. Virchow regarded the new cells as proliferated fibroblasts, but placed the lesion midway between the strictly productive process and purulent inflammation.

(*b.*) The first event after the lodgement of the bacillus in a tissue is the exudation of round cells (lymphocytes) followed later, and only on the outskirts of the lesion, by a growth of epithelioid fibroblasts. This view is held by the French school, Leclainche and Montane, Lecredde, and by Bollinger.

(*c.*) The first effect of Bacillus mallei on the tissues is a necrosis of tissue cells, followed by exudation of mononuclear

and polynuclear cells, and often of blood and fibrin. This opinion is maintained by Tedeschi, Csokor, and Wright.

From a study of the literature on the histology of the lesions of glanders it seems evident that many observers have attempted to draw too rigid conclusions from the material under observation, while more or less completely ignoring the definite observations of others.

Thus one finds attributed to Baumgarten and many other astute observers the statement that giant cells do not develop in the glanders nodules. They were, however, a prominent and characteristic feature of the lesion in the present case. Unna states that no diapedesis of red cells occurs, which was rather strictly true of the cutaneous lesions in the present case, while elsewhere widespread extravasations of blood were present. Ferrari states that in the blood the bacilli are always found within the leucocytes, which can hardly be granted for a violent form of septicemia, and did not prove to be true in sections of blood in the present case. Many observers state that in the tissues the bacilli are mostly extra-cellular, and many others encountered material in which, as in the present case, the intra-cellular grouping of the bacilli was prominently noted. It appears that the course of the inflammatory process in glanders must vary a great deal according to the virulence of the infection, the susceptibility of the organism, and the structure of the tissue involved, and that Bacillus mallei may prove a strict tissue parasite or may actively invade the blood, that hemorrhage occurs in some cases and some tissues and not in others, that giant cells may be absent, that the location of the micro-organisms may be intra- or extra-cellular.

Regarding the essential nature of the inflammatory reaction excited by B. mallei the same variations seem to occur, and to indicate that the reaction may be principally and originally necrotic, or exudative and necrotic, or productive and necrotic.

It appears that many of these variations may be controlled at will. When one rubs up a killed culture in a mortar, and injects moderate amounts of the emulsion into

the skin of a guinea-pig, one will obtain, as did Mayer and others, principally productive lesions. When, on the other hand, one injects considerable quantities of fresh blood serum culture of great virulence into the peritoneal cavity or circulation of a guinea-pig, actively exudative and necrotic lesions have always been obtained. The acute disease in man seems to follow the latter type.

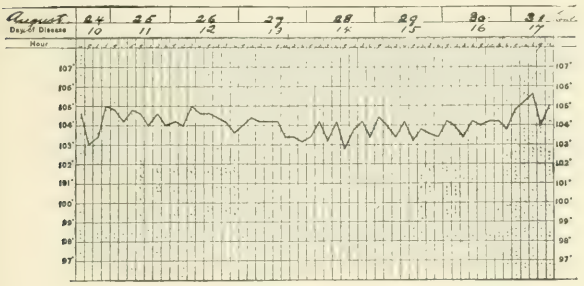
In the present case many of the lesions were purely exudative and necrotic and any tendency to a proliferative process was entirely obscured by the violence of the reaction.

These features were especially prominent in the muscles, where the initial position of the necrotic process was indicated by the following characters: (1) The dissolution of the muscle fibres, which was often extensive and sometimes complete before any trace of proliferation of fixed tissue cells could be detected. (2) By the presence of hemorrhage from rupture of necrosed capillaries which preceded other changes in the endomysium of muscle fibres. (3) By the comparative absence of local leucocytosis in early lesions. (4) By the sudden comminution of the exuded cells. (5) By the narrow margin which separated necrotic exudate and tissue from practically unaffected tissue. Similar purely necrotic and exudative lesions have been described by Wright in the livers of guinea-pigs.

In most of the lesions an exudative element resembling that of a severe pyogenic process was present in the form of excessive fibrinous deposits in and about the necrotic zone. As noted by several writers, the nodules were often surrounded by a zone of fibrinous exudate, which in the lung sometimes filled a complete circle of alveoli about a small necrotic area. Likewise many polynuclear and mononuclear leucocytes were usually seen in the pulmonary lesions from which, however, Staphylococcus pyogenes aureus was isolated.

In spite of the undoubted occurrence of processes of the above type there were many features of the lesions even in this very acute case which support Baumgarten's view that there resides in the glanders bacillus a special tendency to

induce proliferation of fixed tissue cells and which must throw this micro-organism in the general class with B. tuberculosis.



This relation is strongly suggested by (1) the gross appearance of the glanders nodules, which much resemble miliary tubercles, and has led to the classification of the disease with the infectious granulomata. (2) The absence of general and any marked local leucocytosis must stand as evidence of the same relation, as showing that the characteristic qualities of B. mallei are different from those of all active pus-producing micro-organisms. (3) The microscopical study of the lesions in the lungs of the present case showed absence of any considerable proportion of polynuclear leucocytes and the presence of many large mononuclear cells, which sometimes exhibited the epithelioid form as in miliary tubercles although necrosing too rapidly to permit of satisfactory conclusions on their form or regarding their origin from endothelia or fibroblasts. (4) The presence of large numbers of giant cells recall the giant cells of entirely different appearance, but probably of somewhat similar significance in the miliary tubercle. (5) Although plasma cells were present in the outskirts of most lesions in connective tissue, they were not more numerous than about many purulent lesions excited by the streptococcus or staphylococcus pyogenes.

From these considerations and from the observations that have been so often repeated on the action of less virulent cultures on animals, the writers conclude that the glanders bacillus tends strongly to induce a productive lesion somewhat similar to the miliary tubercle. This tendency appears to be much less pronounced than with the tubercle bacillus, while the necrotic and exudative tendencies of the glanders bacillus are much more prominent than with the tubercle bacillus, and purely necrotic lesions are observed under some conditions. These are practically the same conclusions as those reached by Johne, who finds that there is no single type of lesion which the action of the glanders bacillus invariably follows.

The septicemic character of the present case sets it off at an extreme distance from the usual character of the process excited by the glanders bacillus which has been defined by Baumgarten as a strict tissue parasite. Not a few cases of the same type in man have, however, been described which would seem to show that the human subject is one of the most susceptible of animals to this infection.

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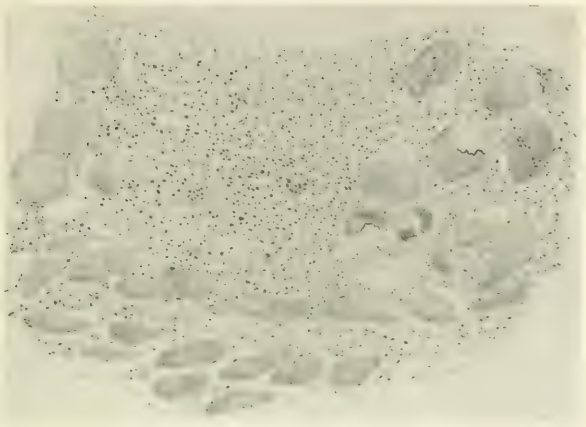

Fig. 1.

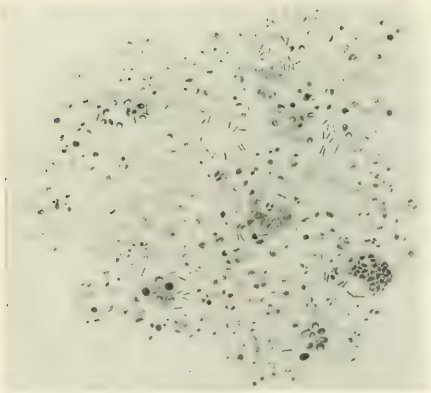

Fig. 2.

Coltman and Ewing. Glanders.

Coleman and Ewing. Cutaneous lesion of Granuloma.

DIFFERENTIATION OF MONKEY AND HUMAN BLOOD BY THE SERUM TEST.

JAMES EWING, M.D.

At a previous meeting of the Pathological Society, I presented the results of some experiments which tended to show that, to get any specific result in the differentiation of animal bloods by the precipitation of albumens by specific anti-sera, one must consider not only the dilution of the blood to be tested but even more carefully the dilution of the test-serum. In those experiments it was shown that in dealing with a very active beef-rabbit serum, one could obtain precipitates with quite a variety of bloods other than beef, if the test-serum was not diluted more than 1 to 5. When, however, the beef-rabbit serum was diluted 1 to 30, only beef and goat blood reacted while at the dilution of 1 to 50 goat blood failed to react, while the beef blood still gave a prompt precipitate. The dilution of the blood to be tested, if under 1 to 500, made comparatively little difference. These results were very similar to those previously obtained with the same method by Kister and Wolff (*Zeit. f. Hyg.*, Bd. 41) but quite different in some minor respects.

I have since then attempted to see to what extent monkey blood of various accessible types reacts to humanized sera. For this purpose I obtained blood serum from four monkeys representing three different breeds and have tested these sera with four different strains of humanized sera. The monkeys tested were two Java (*Innus cynomolgus*), one *Macacus rhoesus*, and one baboon (*Cynocephalus ursinus*). The test-sera used were two rabbit sera called No. 1 and No. 2, prepared by Dr. Strauss in the Cornell Laboratory, one rabbit serum called No. 3, prepared by Dr. Payne in the Laboratory of the Department of Health, and one chicken serum, called No. 4, prepared by myself. I am greatly indebted to these gentlemen for the supply of these sera.

The strength of the sera may be indicated in various ways, perhaps best by the highest dilution in which they yielded a flocculent precipitate in human blood, diluted 1 to 50 or 1 to 100, within three hours or less.

The sera gave flocculent precipitates in one month old human serum, as follows:

Test serum	Dilution of tested serum	Dilution of test-serum	Time
No. 1	1-100	1-50	30 min.
No. 2	1-100	1-50	3 hours
No. 3	1-100	1-200	1 hour
No. 4	1-100	1-50	1½ hours

Serum No. 3 (Dr. Payne's) was extremely active, giving an instantaneous turbidity when added in a dilution of 1-100 to human serum diluted 1-1000. Serum No. 4 (Chicken serum) was distinctly more active than No. 1 or No. 2.

The result with the use of these sera on monkey serum was briefly as follows:

1. *Baboon Serum.* In all dilutions of test sera Nos. 1, 2, and 3, up to and including 1-30, the baboon serum gave flocculent precipitates or turbidities within three hours, which were indistinguishable from many reactions encountered in testing known human blood. This was specially true with serum No. 3 which precipitated baboon serum about as actively as human, up to 1-20, and gave a very slight flocculent precipitate in three hours, when added in dilution of 1-40 to baboon serum diluted 1-200. These precipitates were, however, much less marked than in the corresponding human controls. Even with the active serum No. 3 baboon blood in any dilution failed to yield a flocculent precipitate in three hours when the test serum was diluted 1 to 50. The reactions with sera Nos. 1 and 2 were less marked, and the pronounced turbidities occurring, usually failed to become flocculent in three hours.

2. *Rhoesus Serum.* The reactions with this serum were almost identical with those obtained with the baboon. Sera Nos. 1 and 2 failed to give flocculent precipitates in any but the earlier dilutions (1-10, 1-20) within 3 hours, although causing prompt and distinct turbidities. Serum No. 3, however, gave abundant flocculent precipitates in three hours or less, up to dilutions of the test serum 1-40, beyond which the turbidities failed to become flocculent in three hours, or more. The dilution of the monkey serum if not beyond 1-200 made very little difference in the results.

3. *Java Serum.* This serum reacted more like human blood than did either of the others. With serum No. 1 it gave distinct turbidities up to a dilution of test-serum 1-40, but the turbidities failed to become flocculent. With serum No. 3, a flocculent precipitate was obtained in 90 minutes, tested serum 1-100, test-serum 1-80. With test-serum 1-100 distinct turbidities occurred promptly but failed to become flocculent. With test-serum 1-200 all trace of reaction failed although in the human control a flocculent precipitate occurred in one hour.

From the results with these humanized rabbit sera it appears to be possible to distinguish from human blood, that of certain monkeys by using high dilutions of test sera, the strength of which has been accurately calibrated. The strong sera No. 3 gave the most satisfactory results, since in high dilutions (1-100, 1-200) it failed to react with monkey blood, while still actively precipitating albumens of human blood. With all lower dilutions, however, this serum gave turbidities or precipitates in both bloods, which would not permit the separation of the monkey from the human.

The suggestion arose that an animal more distantly removed from man, might yield a more selective serum. An ordinary hen was therefore treated with 6 intraperitoneal injections of 10 c.c. of defibrinated placental blood, at intervals of 2 to 3 days. Last injection February 5th, serum drawn February 9th. This animal did not react to these injections by any visible symptoms. Its serum, previously inert, was found to precipitate the above specimen of old human serum rather actively, as shown in the table.

When tested on Java and Rhoesus serum it failed to give flocculent precipitate in 18 hours in any dilution and gave only the very faintest turbidities in the earliest dilution, 1-10, beyond which the monkey serum remained clear till developing bacterial growth.

This single experiment does not, of course, demonstrate that all humanized chicken sera will prove so selective, and I have not had time to repeat the experiments with other chickens. In any event it would seem that the chicken may be recommended for this class of experiments. It is easily handled, is cheap, receives injections without apparent reaction, yields blood freely from superficial veins in the wing, stands hemorrhage well, and is apparently immune to most ordinary infections.

I conclude from these experiments that the blood of certain monkeys can be separated from human blood under suitable conditions. One must know accurately the strength of the test-serum used. The stronger the serum, the greater is the danger of confusing monkey with human blood if slight dilutions are used, while with high dilutions the strong sera yield flocculent precipitates long after monkey blood has ceased to react. Very strong serum must be diluted at least 1-100, better 1-200, and confidence placed only in flocculent precipitates occurring within three hours or with a certain promptness.

Humanized chicken serum seems to be extremely selective for human blood and shows less antagonism than humanized rabbit serum against monkey blood.

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PRECIPITINS AND THEIR MEDICOLEGAL USE.

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SCOPE OF DEMONSTRATED PRECIPITINS.

Precipitins for Albumins of Blood.—The original observations from which began the development of the medicolegal serum test for blood were those of Kraus, 1897, who obtained albuminous precipitates in filtered cultures of *Bacillus cholerœ, B. typhosus,* and *B. pestis,* by the addition of homologous immune sera, and that of Tschistovitch who obtained a precipitate in eel serum by means of anti-eel serum from the rabbit.

Very soon contributions from many sources demonstrated the fact that most but not all animals develop antisera with precipitating properties (precipitins) against most alien bloods. Bordet produced in a rabbit a precipitin for chicken blood, and pointed out the distinction between the precipitating and the agglutinating and hemolytic actions of such antisera. Uhlenhuth secured in rabbits precipitins for the blood of the chicken, beef, and man; Wassermann, Schutze, Stern and many others studied the actions of the precipitins of humanized rabbit blood. Nuttall and Dinkelspiel prepared several blood precipitins, including those obtained by treating rabbits with the blood of the dog, sheep, beef, horse and man. Nuttall later obtained, apparently in the rabbit, precipitins for the blood of the fowl, pig, alligator, turtle, frog and lobster. Grünbaum prepared antisera for the blood of the chimpanzee, orang and gorilla, in animals not stated. Von Dungern experimented extensively with precipitin for crab's blood (*Maja squinado.r* and *Dromia vulgaris*), developed in rabbits. One of us has found that the chicken produces an active precipitin for human and rabbit bloods. Noguchi found the normal serum of several cold-blooded animals to contain precipitins for other cold-blooded animals and for horse serum, while leaving somewhat un-

certain the possible increase of these agents by immunization of such cold-blooded animals. Metchnikoff, Mesnil and v. Dungern, failed to increase natural or produce new precipitins in various cold-blooded animals. DeLisle, working with eel blood, failed to immunize the guinea-pig against this serum as the animals invariably died, but, with more difficulty than Tschistovitch describes, he produced in the rabbit an active hemolysin and precipitin for eel blood, and in the eel hemolysin and precipitin for rabbit blood.

In the experience of the above observers it has not always been possible to obtain in every animal, a precipitin or hemolysin for every other animal blood. Thus Bordet failed in this attempt with guinea-pigs treated with rabbit's blood, but one of us recently secured in guinea-pigs, an active hemolysin and precipitin for rabbit's blood. Nolf failed to obtain a precipitin from pigeons treated with chicken blood, and Nuttall from rabbits treated with cat's blood. These results, in the case of closely related animals, must be referred to the lack of antagonism between the blood of such animals, and in the other case to the excessively poisonous effects of the eel serum.

It may here be noted that Friedenwald found that when an animal is injected with the serum of a closely related animal, the albumins pass rapidly out of the system with albuminuria, while the more distinctly alien blood is retained in the system apparently absorbed by the body cells.

Precipitins for Milk.—From the earliest stages of the study of blood precipitins, investigations have been directed to specific sera antagonistic to other body cells and other forms of albumins. Bordet first produced in rabbits by injections of sterile cow's milk a serum precipitating in slight dilution the albumins of such milk. Fisch prepared specific precipitins for the casein of cow's milk, which failed to affect either goat or human milk. He also injected an emulsion of udder cells and obtained a specific precipitin for the milk of the same animal, and concluded therefrom that milk represents a solution of the gland cell albumins and not merely a filtration through the cells. Wassermann and Schutze obtained in rabbits precipitins for human, goat's and cow's milk, which in moderate dilution proved highly specific for each variety of milk. Hamburger obtained specific precipitins for both the albumin and the casein of cow's milk, both of which sera acted similarly on beef serum, but the serum ob-

tained after injections of beef blood, had no effect on cows milk. This result indicates that both serum and milk contain receptors which produce precipitins for serum albumin, but milk contains other receptors which develop a special precipitin for the albumin of milk.

That other tissue cells contain the milk-coagulating receptor is indicated by the results of Meyer and Aschoff, who obtained milk coagulins by injections of various tissue cells. While some reports indicate high specificity for the milk coagulins, Moro found that the precipitin developed by cow' milk precipitates also goat milk. Careful dilution of the test serum has apparently not been observed in this field.

Precipitins for Muscle and Bone Albumin.—Schutze produced a specific precipitin for human muscle, which was inert on human albuminous urine or blood serum, but proved hemolytic. Cooked muscle also yielded a similar precipitin, as did also an albumin separated chemically from muscle tissue. Jez and Uhlenhuth have since employed this biological method for the detection of different meats, and Schutze has applied it with some success to the differentiation of bones.

Precipitins for Albuminous Exudates.—Many observers have found that albuminous exudates in pleuritic, ascitic, and hydrocele fluids and albuminous urine, are almost as active in developing precipitins for blood serum as is the blood itself. As such fluids are readily obtainable they are often employed instead of blood for the preparation of blood precipitins for medicolegal purposes, but there are various reasons for avoiding such expedients.

LeClainche and Vallée first employed intravenous injections of urine containing one-half grain of solid albumin per liter. The rabbits suffered considerably from the treatment but after three months their sera actively precipitated urine containing serum albumin. They gave a slight precipitate with urine containing much globulin, but were inert with human blood serum, or serum from the horse, donkey, sheep and beef. Mertens found that injections of albuminous urine yielded less precipitin for the albumins of the urine or blood than did injections of blood. Zuelzer, who also employed highly albuminous urine, concluded that the injected urine must contain some blood albumins but not these exclusively. Biondi found that injections of kidney tissue free from

blood yielded precipitins both for blood and for albumins in the urine, while Schattenfroh found that injections of albumin-free urine, if not heated, produced blood hemolysin and agglutinin but no precipitin. A comparison by Dieudonné of the activity of the serum yielded after injections of albuminous urine, pleuritic exudates and blood serum, showed a considerable difference in favor of the blood serum.

Specific Precipitins for Albumins, Globulins and Peptones.—Several observers have endeavored to separate the different proteids of blood and to test their actions apart from the mixtures in which they naturally occur. Nolf separated the plasma from the cells of chicken and dog blood and secured in each case precipitins only from the animals receiving injections of the plasma. He precipitated the globulin twice by saturation with magnesium sulphate and washing, and separated the albumins from the dialized filtrate by the addition of one per cent. Ac. adding NaCl and sterilizing at 56° C. for eight days.

Rabbits treated with the globulin developed a precipitin while those receiving the albumin did not. That the substance precipitated (precipitum) is a globulin he concluded from the fact that the active serum had no effect on solutions of serum albumin but gave abundant precipitates in solutions of globulin.

Biondi failed to obtain a precipitin by injections of serum albumin, but succeeded by the use of the globulin precipitated by $MgSO_4$.

In reference to these experiments it may be remarked that the serum-albumin may have been greatly altered in character during its separation.

Myers secured from rabbits precipitins for crystallized egg-albumin, sheep globulin, beef globulin, and Witte's peptone, by injections of each of these substances. Rather numerous control tests showed these precipitins to be highly specific but not absolutely so. The peptone serum was peculiar in losing much of its activity when heated to 56° C., and on being reactivated by normal rabbit serum, while all the sera agglutinated various blood cells. Pick and Spiro also claim to have secured a precipitin for albumoses.

Uhlenhuth injected chicken egg-albumin dissolved in salt solution and obtained a powerful precipitin which had a slight action on pigeon egg-albumin, and resisted heating to 60° C. for one hour. Feeding the animal for twenty-four days with the same albumin also developed the

precipitin, but Moro and Hamburger failed to obtain any lactose serum by forced feeding of young animals by milk. Obermeyer and Pick and Umber separated the different albumins and globulins of the white of egg, and while they all caused the development of precipitins, these precipitins were not specific for the different proteids of the egg. Modica and Corin also obtained precipitins by injections of globulin precipitated by magnesium sulphate. Corin secured the globulins from cadaver blood and ascitic fluid, and demonstrated that the precipitin is also a globulin, and he recommends its preservation in the form of a dry powder containing magnesium sulphate. Strong solutions of this powder he found more active than the fresh serum from which it was derived.

Somewhat dissimilar results were obtained by later observers. Rostocki, Landsteiner and Calvo, found that the serum of rabbits immunized to horse globulin precipitates both globulin and albumin of horse blood. In Michaelis' hands injections of pure globulin gave a serum precipitating globulins only, while injections of pure albumins gave a serum precipitating both albumins and globulins. As he obtained the globulin by partial saturation with ammonium sulphate, his experiments are not exactly comparable with those in which the globulin was precipitated by magnesium sulphate. He failed to obtain a precipitin from Merck's egg-peptone or Riedel's peptone, and points out from the observations of Buchner and Geret that Myers' peptone precipitin probably represents a precipitate of barium sulphate. Tschistovitch also failed to obtain a precipitin for peptones. Ide and Leblanc both report that they have secured specific precipitins for pure albumin and pseudoglobulin of beef blood, and Hamburger claims that the casein and albumin of milk may be separated by their specific antisera.

Obermayer and Pick[2] report that beef serum heated to 60° to 70° C., which destroys its reaction to the antiserum, is capable on injection into rabbits of developing a serum which precipitates not only fresh beef serum but also beef serum heated to 60° to 70° C. By employing such heated sera they secure what may be called a polyvalent serum which may possibly prove of value in testing blood which has been subjected to heat. Likewise beef globulin carefully oxydized at room temperature by potassium permanganate and thus deprived of its capacity to react to nor-

mal antiserum will devolop a serum which precipitates the oxydized globulins and to some extent the fresh globulins.

The observations of Klein indicate that the precipitins produced by the albumins of the red cells and those produced by the albumins of the serum are not identical.

Finally it should be mentioned that Camus and Bordet obtained precipitins by injections of fibrin.

From the above data it may be concluded that the most active principle of blood in exciting the development of precipitins, is the globulin. Possibly it is exclusively the globulin which possesses this property, but further evidence on this point is required. Peptones, have so far failed to develop precipitins.

Precipitins for Vegetable Albumins.—From the reports of Schutze and Kowarski, it appears that precipitins have been obtained for vegetable albumins, the former using *"roborate,"* while Kowarski's precipitin for wheat albumose acted also on albumoses of rye, peas and barley, but not on that of oats. Connected with the precipitins for vegetable albumins is the entire field of specific bacterial precipitins.

The above series of observations on the great variety of already demonstrated precipitins has been briefly collated for the purpose of presenting the more important results in compact form, but especially to emphasize from the medicolegal standpoint that the existence of specific precipitins is not an isolated scientific fact. but that the discovery of these bodies has brought to light a very comprehensive and entirely new principle in biology.

By virtue of their complex molecular constitution the various members of the different classes of proteids of the animal and vegetable kingdoms are found to differ from each other, not merely in some of their grosser chemical reactions, but by a new series of properties of obscure character indeed but no more surprising and no less definite than that recognized in meats by the sense of taste. Moreover, Ehrlich's conception of the mode of origin of these precipitins and other antibodies has offered a logical explanation of these peculiar phenomena and removed much of the obscurity surrounding them. The factors concerned in the precipitin reaction may now be considered in detail.

NATURE OF THE PRECIPITIN REACTION.

For the full discussion of the principles gov-

erning the development and action of precipitins, according to Ehrlich's conception of them, the reader is referred to the original articles of Ehrlich and to the summaries of Ritchie in English and Aschoff in German. Here it need only be said that the precipitins are cast off cell receptors (side chains) of Order II, possessing a stable haptophore group uniting with the coagulable molecule and a more or less labile zymophore group, which exerts a precipitating action on the affected cell or molecule.

As to the nature of the process by which the large proteid molecule is precipitated from solution, recourse must be had to the laws governing variations in surface tension.

The bodies concerned in the development and action of precipitins may be divided into three classes: (*a*) Those exciting the development of the precipitating substance of the serum; (*b*) those constituting the precipitating substance; (*c*) those constituting the precipitate and affecting its formation.

(*a*) Substances Exciting the Development of precipitins.—From the foregoing section it will be seen that this property has been shown to reside in most classes of proteids both animal and vegetable, including serum globulin, and the globulins separated by fractional precitation by ammonium sulphate, [serum-] and egg-albumins, albumins of tissue cells, vegetable albumoses, and gelatine; while mucins and peptones have not yet been included in the list.

From what had previously been shown, regarding bacterial immunizing substances, it appeared likely that of all of these substances the globulins were most active in developing precipitin, and this conclusion has been strengthened by the results of many observers working with blood precipitins. Nolf and Biondi failed to obtain any precipitin from injections of pure serum albumin, while in the reports of many others, who succeeded in this attempt, there has been little effort to compare the activities of the serum on the respective substances injected.

(*b*) What Constitutes the Precipitating Substance.—As has been previously shown for the majority of the specific antibodies, the precipitins belong chiefly or exclusively in the class of globulins. Yet the studies on which this generally accepted conclusion is based were principally concerned with the antibodies for injected globu-

lins, so that it is perhaps too early to say that all precipitins belong in the class of globulins.

Corin by saturation with Mg. sulph. at 30° C., precipitated the globulins of serum prepared against defibrinated blood and found a 4.5-per-cent. solution of this precipitate more active than the original serum. Michaelis precipitated the globulins by partial ($^3/_{10}$) saturation with ammon. sulph., and found the precipitate to contain practically all the active properties of the serum, and that the dissolved precipitate acted only on globulins, not on serum albumin. Ziemke found the globulins precipitated by ammonium sulphate, and dissolved in soda solution, less active than the fresh serum and therefore could not fully endorse the suggestion of Michaelis to precipitate the globulins in order to preserve the active principle of the serum. Moreover Pick found that the precipitated globulins of typhoid serum deteriorated much more rapidly with age or by heating than did the original serum. The precipitin belonging in the class of Ehrlich's receptors Order II, as already mentioned, contains a more or less labile zymophore group, which may suffer deterioration while the haptophore or cell-binding group is still intact. Thus arise in a variety of ways the inert forms of precipitins called precipitoids.

(c) What Constitutes the Precipitum.—That the precipitum from blood serum is a globulin was first claimed by Nolf, from the fact that his antiglobulin serum precipitated a solution of that globulin, but not one of serum albumin. LeBlanc came to the same conclusion from the analysis of the precipitum obtained from solutions of hemoglobin. P. Müller found the precipitum from milk, resulting from the action of its antiserum, to consist of casein. The precipitum also contained the precipitin itself, which he succeeded in extracting in active form by strong acetic acid. Von Dungern precipitated by its antiserum the blood of *Octopus vulgaris* which contains a single albumin, hemocyanin, characterized by a 38-per cent. content of Cu. In the precipitum he recovered a considerable portion of Cu. The dissolved precipitum was non-dialyzable.

The general properties of the precipitum are those of albuminous substances. Tschistovitch found the sediment of eel serum insoluble in water or alkaline carbonates or neutral salts, but soluble in dilute acids and alkalies. It was non-toxic to

rabbits. Bordet, Michaelis and others also proved the solubility of the precipitum in dilute acids and alkalies. Uhlenhuth dissolved blood precipitum in NH_4 OH and H_3 PO_4. On the other hand there is evidence to show that the substance attacked by the precipitin is not necessarily a proteid, but may be merely associated with or derived from the proteids. Thus Obermayer and Pick found that the precipitable substance of white of egg may be altered by frequent crystallization and by digestion with pepsin, so that it no longer gives the biuret reaction, although still precipitating with antiserum. Jacoby and Haussman also digested ricin and abrin with trypsin, destroying the biuret reaction, but the usual precipitate followed addition of antiricin. Their results do not indicate, as v. Dungern says, that the molecule uniting with the precipitin was not originally connected with the natural albumins of the solution, but they at least show that these albumins may suffer considerable alteration without losing their affinity for precipitin, and they have practical bearing on the results of the precipitin test in decomposed blood.

The relative proportions of precipitin and precipitable substance entering into the precipitate may be estimated in approximate degree in the light of certain observations. Linossier and Lemoine found that 25 parts of an active humanized rabbit serum were completely neutralized by one part of human serum, while 200 to 300 parts of rabbit serum was necessary to remove the precipitable substance from one part of human serum. These results do not justify more than the conclusion that the precipitin is usually vastly less abundant than the precipitable substance. From the investigations of Eisenberg and Volk on typhoid agglutinins, it is evident that the absorptive power of agglutinins varies with their dilution, being greater the higher the dilution. This same rule seems to apply to the precipitins and has important bearing on the technic of the serum test.

ARTIFICIAL AND SPONTANEOUS CHANGES IN PRECIPITIN SERUM.

Effects of Age.—All observers have found that precipitin sera rather rapidly deteriorate with age, and recommend careful preservation in sealed tubes, on ice, and in the dark. There are, however, very few reports regarding the duration of active properties of serum thus preserved, and the significance of such reports vary greatly with

the original strength of the serum. Strube and Uhlenhuth both found serum still active after three months, the latter having preserved his specimen with .5 per cent. carbolic acid. The addition of chloroform commonly recommended prevents bacterial growth, but does not save the serum from gradual deterioration. From such serum Ziemke reports considerably diminished activity after more than three months. Okamoto found his chloroformed serum sealed in glass tubes still active after one month, but inert after two or three months. Corin and Stockis found the dog globulins still active after two months, but do not report on their later condition. Chirokikh found that filter paper soaked in the serum yielded an active precipitin solution after one month.

We were unable to detect any loss of activity of a powerful humanized serum after ten days on ice without preservative. The serum of two beef rabbits kept on ice with chloroform showed no distinct change, one after sixty-three days, the other after eighty days. A powerful humanized serum kept ten months on ice with chloroform, and which showed a greenish discoloration and had probably become somewhat concentrated by evaporation, still gave an abundant flocculent precipitate at dilution 1 in 30 in two hours in human serum 1 in 100. Controls on heterologous blood were negative in all the above reports. We agree with Ziemke that no method of preservation can replace the advantage assured by working with fresh serum.

Effects of Heat.—Many precipitins have proved comparatively resistent to heat. Jacoby found antiricin active after heating for two hours at 60° C. Myers exposed the antisera of egg albumins and globulins to 56° C. for one-half hour without altering their activity. Tschistovitch found his anti-eel serum less active after heating to 60° C., and inert after heating to 70° C. Uhlenhuth's antiserum for egg albumins was unchanged by heating for one hour at 60° C. Leclainche and Vallée heated their antiserum from albuminous urine for two hours at 58° C., without diminishing its activity. Michaelis destroyed his blood precipitin by two hours' exposure at 68° C. Biondi concluded that blood precipitin serum begins to lose its activity when heated to 65° to 70° C., but if dry resists heating up to 130° C. Eisenberg found the dry globulin active after heating one-half hour at 100° C., but inactive af-

ter the temperature reached 130 to 135° C. One of our potent beef rabbit sera was rendered almost entirely inert by heating in a water bath at 55° C. for fifteen minutes, while not a trace of reaction occurred after heating to various moderate temperatures above 55° C. A humanized rabbit serum, however, lost little activity by heating to 60° C. for fifteen minutes, but became practically inert after fifteen minutes' exposure at 65° C. Michaelis has studied extensively the inhibition of precipitin reaction by heated precipitin serum and other agents. He finds that serum heated to 72° C. loses its precipitating properties and also strongly inhibits the action of fresh precipitin serum subsequently added. In smaller quantities the heated serum merely lengthens the time required for the transformation of turbidity into flocculent precipitate. An excess of precipitable substance also prevents the formation of precipitum and rapidly dissolves it when formed. Any albuminous solution in considerable concentration inhibits the action of all precipitin sera.

Action of Chemicals.—Many salts, as NaCl, $MgSO_4$ $(NH_4)_2SO_4$, have no deleterious action on precipitins, and are employed in their separation. Antiricin withstands the action of dilute H_2SO_4, and NaOH, at 37° C. for one-half hour, and is not altered by pepsin and trypsin (Jacoby), but Michaelis destroyed blood precipitin by digestion for one hour with pepsin and HCl, although each of these agents alone was inert. He also removed the calcium from the active serum by oxalic acid without affecting its activity. Pick in his comprehensive studies of typho-precipitin from the horse found it to be destroyed by digestion with pepsin or trypsin, by strong acidification, by urea, by a "few drops" of formalin, while the presence of a remnant of ammonium sulphate rendered the precipitated globulins inactive; the serum was less active in the presence of .8 per cent. salt than in some smaller proportions of salt, and calcium salts played little or no part in the reaction.

Precipitoids.—The spontaneous development or artificial production from precipitins of a substance devoid of precipitating powers, but still capable of chemical union with the precipitable molecule has been observed for many of the precipitins and these substances are called *precipitoids*.

According to Ehrlich's conception, they are the precipitins which have lost the zymophore group,

while retaining the haptophore group, of the complex molecule. Their development and action are illustrated in experiments with lactose serum by P. Müller, who found that heated lactose serum, while itself no longer precipitating the albumins of milk, prevents the action of rennet and fresh lactose serum ,but if the coagulins are first removed heating does not cause the serum to develop the powers of an anti-coagulin.

CHANGES IN THE PRECIPITABLE SUBSTANCE AND THEIR EFFECTS.

The precipitable substance may be exposed to a great variety of influences, natural and artificial, without losing its susceptibility to the precipitin, which, however, is lost when the albuminous molecule is dissociated or loses its receptor for the precipitin.

Effects of Heat.—Tschistovitch secured less precipitum in eel sĕrum if heated to 58° C., and none after 80° C. Leclainche and Vallée found that heating albuminous urine two hours at 58° C., diminished its response to antiserum more than similar treatment of the antiserum itself. This somewhat anomalous result may perhaps be referred to the disturbing action of urea (cf. Pick). Nuttall concluded that blood precipitin and the precipitable substance are about equally resistent to heat, as dried serum heated one-half hour at 100° C. still reacted, while serum diluted 1 in 100 was unaffected at 55° C., but failed to react after heating to 100° C. Eisenberg was unable to destroy the reaction of dried bacterial precipitins or precipitable substance until the temperature reached 130 to 135° C., for one-half hour. Ferrai reports partial or complete loss of reaction from blood stains heated to 130° C. for one hour, 140° for twenty minutes, 150° for ten minutes, and 160° for five minutes. Biondi places the limit of resistance to heating of dried blood at above 130° C., finding some specimens to react after exposure to this temperature. Okamoto obtained distinct coagula from blood stains heated one-half hour at 50° and 100° C., but negative results with stains heated one hour to 150° C. Obermayer and Pick[2] find that beef serum heated to 60° or 70° C. not only fails to react to its antiserum, but also inhibits the action of precipitin upon fresh serum when added to that serum in certain proportions. Corin finds the

precipitin reaction favored between temperatures of 37° and 50° C.

Drying seems to preserve indefinitely the special properties of the precipitable albumins of blood, but not without some deterioration. Thus Ziemke secured a definite reaction from an extract of the stomach of a ten-year-old mummified cadaver, from dry stains two, nine, fifteen and twenty-five years old, from five-year-old stains on rusty knives, from an eight-year-old washed stain, and from blood one and three years in earth. Yet most of the old stains did not react strongly, yielding only turbidity without flocculent precipitate, and one ten years old, on a shirt, failed to react at all.

Uhlenhuth obtained a reaction (turbidity) in one minute with blood stains six to twelve years old, and Biondi secured definite reactions with stains one to fifteen years old, but failed with one twenty years old. Graham-Smith and Sanger obtained slight or marked turbidities in five to sixty minutes with nearly all of 29 stains three to thirty years old, from the Scotland Yard Museum, 17 of which came from knives which had been smeared with oil to prevent rusting. These authors found that fluid sera and egg albumin nine and fourteen months old had lost respectively 7 per cent. and 12 per cent. in precipitum, while one specimen of egg albumin nine months old had lost 33 per cent. Okamoto, 1903, obtained very feeble reactions from dried blood on wood and limestone dating from 1867, and 1874, but abundant precipitate from a dry blood clot preserved from a venesection in 1874. Some observers report reactions from decomposing blood, and from mixtures of several bloods. Uhlenhuth passed through the Berkefeld filter extracts in normal salt solution of several specimens of alkaline decomposing blood, from man and animals, and obtained reactions, prompt turbidity, only with corresponding antisera. Soapy washings of blood stains, urine, blood frozen two weeks, and carbonic oxide blood, all gave the same result. Ziemke secured reactions from recently decomposed blood and from a three-year-old decomposed cadaver. Graham-Smith and Sanger grew various putrefactive organisms thirty-six to fifty days in pleuritic exudate, beef serum, and horse serum, and found usually a decrease of precipitum from 20 to 50 per cent., but with some putrefactive species (unnamed), and in all the specimens of horse serum the precipitum was in-

creased, once as much as 24 per cent. All were slightly alkaline or neutral in reaction, and control tests were negative. From Okamoto's experiments it must be concluded that the presence of ammonia in old or decomposing blood may prevent precipitation.

Effects of Acids on the Precipitable Substance.—As the precipitum is soluble in rather weak acids and alkalis any great variation from a neutral reaction of the tested solution must be avoided. According to Linossier and Lemoine and Rostocki a slight acid reaction favors precipitation, while alkalinity hinders it. These observations accord with Pick's conclusion that the precipitum most resembles an alkali-albumin. Michaelis finds that human blood precipitum is soluble in dilute acids and alkalis and reprecipitated by neutralization. Graham-Smith and Sanger tested the action in .6-per-cent. salt solution of various acids, sulphuric, nitric, hydrochloric, acetic, oxalic, tartaric, carbolic, pitric, citric, salicylic and chinosol. All of these in dilutions up to and including 1 in 10,000, caused turbidities on addition of test serum, except carbolic and salicylic, which were without effect in dilutions of 1 in 1,000 and above. Some inorganic acids, however, caused very little clouding at 1 in 100, although more active both above and below this dilution. Strong alkalis were less uniform in action, caustic potash and soda caused turbidities in 1 to 1,000 dilution, while sodium carbonate and ammonia were inert at 1 in 10 and above, and were therefore available for neutralization of acid fluids. They found that small quantities of acid or alkali, especially of acid, diminished the quantity of precipitum. They concluded that it is necessary to test the reaction to litmus of all solutions of doubtful source and to neutralize if they are found decidedly acid or alkaline. In testing old blood stains from leather they found the solutions acid, except those from chamois leather, which were alkaline. Neutralization by sodium carbonate was therefore necessary before adding the serum. A thick, polished yellow leather gave a highly acid reaction, and no method of neutralization could be found which permitted the successful use of the test. The destruction of the precipitable substance was referable apparently to the tannin which in dilution of 1 in 1,000 clouds serum heavily. One of us was unable to obtain a specific reaction from human blood which had dried two months on shoes of

a moderately cheap quality of red leather. All of the solutions of the stains in .6 per cent. salt gradually yielded spontaneously an abundant albuminous precipitate without the addition of any serum, and the decanted clear fluid continued to precipitate various sera subsequently added—probably from the presence of tannin. All the solutions were neutral to litmus. Okamoto also reports spontaneous precipitates from solutions of blood stains on leather. Since ammonia and phosphoric acid dissolve the precipitin (Uhlenhuth), the presence of these agents may prevent the reaction.

Action of Salts on the Precipitable Substance.—In order to avoid spontaneous precipitation of albumins a certain proportion of salt must be present in the solvent, since solutions of blood in distilled water slowly become cloudy and deposit a sediment in twenty-four hours. Normal salt solution, .6 to .9 per cent., has proven the best solvent from which spontaneous precipitates rarely occur in twenty-four hours. Linoissier and Lemoine using 1 in 20 solution of blood report that 1 per cent. of salt gives precipitation, while 5 per cent. completely arrests it. On the contrary, Eisenberg and Rostocki find no difference in the quantity of precipitum when the proportion of salt reached 10 to 18 per cent. Graham-Smith and Sanger tested human blood solutions with increasing quantities of salt from .6 per cent. to saturation. At 1 per cent. the precipitum was slightly diminished (12 per cent.), at 2 per cent. of salt content almost the full amount was obtained; at 3 per cent. there was again a diminution, after which the precipitum increased until with saturation the precipitum reached 133.8 per cent. Very different results, however, were obtained with sheep serum. Beyond the fact that normal salt solution is the best diluent, present data do not, therefore, permit us to state rules regarding the influence of salt on the precipitin reaction. The same authors found turbidities to arise on the addition to tested fluids, of sodium and potassium tartrate 10 per cent., sodium acetate one per cent., potassium cyanide one per cent., borax one per cent., while sodium citrate, magnesium sulphate, and potassium nitrate and chlorate, were inert.

Action of Earth on Blood.—Several observers have secured positive reactions from blood mixed with earth, and the effects of such mixtures have been studied in detail by Graham-

Smith and Sanger. They mixed equal quantities of human serum with detritus of chalk, red brick, Pasteur filter, Berkefeld filter, ordinary earth, white brick, mortar, and lime, one specimen of each being kept dry, and one wet, for four days, with the result that there was in all well marked diminution in the quantity of precipitum, average 20 to 30 per cent., while mortar and lime completely destroyed the precipitin substance. They found that the lime in ordinary earth precipitates test serum and must be removed by a current of CO_2 which does not interfere with the precipitin reaction, but that the lime of ordinary earth does not seriously affect the precipitable substance of blood mixed with it.

In this connection the writers may record a complete failure of many attempts to secure a reaction from human blood mixed for three months in earth containing much decomposing vegetable matter. Ziemke's results with certain specimens of blood mixed with earth, one to three years, must also be regarded as negative. Okamoto found that solutions of blood from sand and mortar sometimes gave spontaneous precipitates.

Effect of Antiseptics.—Carbolic acid or chloroform are commonly employed to inhibit bacterial growth in antisera and in tested fluids requiring observation longer than three hours. Yet each of these agents in concentrated form precipitates albumins from serum and gives turbidity if present in proportion of one per cent. or more. Under one per cent. they are inert. Graham-Smith and Sanger tested the action of several antiseptics. Mercuric bichloride and silver nitrate clouded serum at 1 in 10,000 dilution; copper sulphate at 1 in 100,000, formalin, thymol, benzol, toluol, xylol, and ether, at 1 in 100. Lysol and lysoform in high dilutions also produced turbidity.

Results Obtained from Blood Stains on Miscellaneous Materials.—The writers obtained an abundant spurious reaction from blood stains on wall-paper. Controls made from unstained portions of the wall-paper gave abundant precipitates with different antisera, showing that the precipitation resulted from the action of some chemical in the paper. The solutions were neutral. Graham-Smith and Sanger report pronounced spurious reactions from ten samples of wall-paper.

They obtained good reactions from one thick

blood stain on oak, but the test failed with minute stains on cedar and pine. Most observers have encountered no difficulty with stains on wood. One of us was unable to obtain satisfactory results from human blood stains on starched cuffs owing apparently to the presence of dissolved starch in the solution, which gave marked turbidity with various antisera.

Spontaneous Precipitates.—A short experience in the medicolegal use of precipitin sera will seldom fail to include the occurrence of spontaneous turbidities, and precipitates, in solutions prepared for testing. These are usually referable to the presence in the solution of chemicals contained in the material on which the blood is found. Okamoto observed such precipitates in solutions of blood stains on leather, sand and mortar. The writers have observed them in solutions of blood stains on shoe leather and shoe strings, and in one case in which vegetable albumins were dissolved from a rusty knife.

Specificity of Precipitin Reactions.

The first observations of workers dealing with sera of moderate potency and mixing the sera in indefinite proportions rather uniformly indicated that the reactions of precipitins were almost if not quite specific. Yet from the first a certain degree of similarity in the reactions of blood of closely related animals was observed. Bordet found his chicken precipitin to affect also pigeon blood, and Myers' chicken-egg precipitin was active with duck's egg, and his sheep globulin serum affected egg-albumin and ox-globulin. Uhlenhuth's potent hen's egg-albumin serum while active with pigeon egg-albumin, was entirely inert on other varieties of commercial albumins. Leclainche and Vallée found their serum obtained with albuminous urine precipitated human pleuritic exudate but not human blood serum or serum from the horse, donkey, sheep or beef.

Wassermann and Schutze declared in favor of the medicolegal value of the test, on finding that their humanized rabbit serum failed to appreciably affect any one of 23 different animal bloods, except that of the baboon, which reacted more slowly and incompletely than human blood. Stern also found that a humanized serum negative to horse, ox, sheep, and pig bloods, gave a feeble reaction with the blood of three species of monkey. It soon became apparent that the more potent the serum the more pronounced, under the

usual conditions is its effect on alien bloods. Chirokikh and Niedrigailow, using active humanized sera obtained precipitates, in the course of four to five hours, in all of several animal bloods tested.

Nolf's dog blood precipitin, Uhlenhuth's beef and humanized sera, and Wassermann's and Stern's humanized sera, were all reported to be specific for the blood used in the injections, but the exact dilutions were imperfectly stated. Uhlenhuth, not attempting to calibrate the serum, was unable to distinguish from each other the blood of the chicken, goose, duck, guinea-hen and pigeon.

Strube was among the first to point out the activity of humanized sera on other than human blood, finding that a powerful serum precipitating human blood in a dilution of 1 in 5,000 gave a precipitate also in monkey, guinea-pig, chicken, swine, calf, dog, pigeon, and sheep bloods. He speaks of instant turbidity occurring on the addition of this serum to chicken blood in proportion of 1 in 50. This is a very unusual experience. Using a more powerful humanized serum active in dilution of 1 in 20,000 (?) he found no reaction in a series of other animal bloods when the serum was diluted at least 1 in 100 and almost no trace at 1 in 10. He found that human blood was more actively precipitated by horse and beef antisera than by hog antiserum. He fails to state the exact details of these tests but the results were sufficient to point out the necessity of controlling the dilution of the test serum, and of determining by actual experiment what individual peculiarities each serum may exhibit.

Nuttall in an extensive study not yet completed has shown that the reactions with potent sera extend over closely related classes in the animal kingdom. Testing several antisera on the blood of over 500 animals of various classes he finds that a mammalian antiserum affects in varying degree the blood of nearly all mammals, but not without striking exceptions. Such sera however fail entirely to affect the blood of birds or reptiles. A reptilian or avian antiserum, while active in many birds and reptiles, fails to touch mammalian blood. Dealing with mammals he found that humanized antiserum was almost as active on the blood of the high monkeys, *Simiidæ*, as on human blood, less active with the *Cercopithecidæ*, very slightly with the *Hapalidæ* and *Cebidæ*.

Similar results were secured with Grünbaum's

antichimpanzee serum. Antihorse serum precipitated the blood of eight species of *Canidæ*, but was entirely inert with 499 other bloods. Antibeef serum affected the blood of most ruminants. Antipig serum proved unusually diffuse in its effects, clouding the blood of man, monkey, marsupial, bat, and porpoise. Antifowl serum acted more or less on all avian bloods tested, but not on 357 other bloods. A powerful anti-egg serum affected slightly the blood of the alligator. Several reptilian antisera proved highly specific in their subclasses, that of the alligator affecting only the alligator and turtle, out of 500 bloods. Lobster antiserum precipitated lobster and crab blood only, from 503 bloods tested, and frog antiserum had no effect on 508 non-batrachian bloods.

It is important to note in reference to Nuttall's reports that "none of the reactions in heterologous bloods would ever be mistaken for a full reaction such as homologous blood gives." This statement does not apply to the reactions of humanized serum on the blood of higher monkeys.

The attempt to define a specific reaction must consider several factors entering into the reaction: (*a*) The potency of the serum; (*b*) the dilution of the serum; (*c*) the time elapsing; (*d*) the concentration of albumins in the tested fluid.

It has been shown that the more powerful the serum the wider is the scope of its action on the blood of related animals. Thus a feeble humanized rabbit serum in dilution of 1 to 5 may have no effect whatever on any but human and monkey bloods, while a very active humanized rabbit serum in the same dilution may precipitate the blood of several domestic animals. It might, at first sight, appear better, and has been formally recommended by Wasserman, to avoid the use of very potent sera in medicolegal work. On the contrary it has been fully demonstrated by Kister and Wolff, and in the experience of the writers, that the more powerful sera, permitting of rather high dilution, give much more reliable results than can be obtained by weaker sera less diluted. Overimmunized animals whose sera are relatively more potent with heterologous than with homologous blood are theoretically possible, but have not yet been observed.

If one compares the action of a weak serum precipitating human blood only in dilution of 1 in 15 and heterologous bloods in dilution of 1 in 1, with that of a powerful serum potent against human blood in dilution 1 in 200 but increased

in activity on heterologous blood only up to 1 in 10, it will be seen that with the more potent serum the limits between dilutions precipitating human and heterologous bloods are very much greater than with the weaker serum, *i.e.*, as 190 is to 15. Hence the powerful serum may be safely diluted far beyond the point when it can affect heterologous bloods.

Moreover, it is only the more powerful sera which can be relied upon to cause precipitates with the minute quantities of blood frequently encountered in medicolegal work.

Nuttall has studied extensively the grades of reaction of various antisera on related animals and suggested a method of measuring the degree of reaction. This method involves the calibration of the test serum and tested blood and the estimation of the bulk of the precipitate formed after forty-eight to seventy-two hours, beyond which time the precipitin does not vary.

The detailed results thus obtained have not yet appeared, but Smith and Sanger describe the method as follows: By means of an accurately graduated pipette .5 c.c. of a 1 in 21 dilution of serum in .6 per cent. salt solution is placed in a small clean dry test-tube, and .1 c.c. of antiserum added.

After thoroughly mixing, the specimen stands twenty-four hours when the precipitum settles. The supernatant fluid is pipetted off and the precipitum drawn into a capillary tube which is sealed and allowed to stand for seventy-two hours.

The bulk of the precipitum is then read from the tube which is graduated in cubic centimeters. The average quantities of precipitum obtained by adding their antisera to human and beef blood in the above preparations were .0293 c.c. for the human blood and .0233 c.c. for the beef blood. This method is not claimed to escape errors of less than 10 per cent., arising mainly from variations in the quality of the precipitum. It is specially adapted to experimental studies and measuring of blood relations among animal species but does not appear fortunately adapted for medicolegal work.

Kister and Wolff have very clearly shown that by proper dilution of the test-serum, reactions in heterologous blood may be avoided. Working with horse precipitin from the rabbit, they found that when the test serum was added in proportion of 1 in 5 to solutions of serum diluted 1 in 10 or up

to 1 in 320, instant turbidity appeared in sheep, human, beef, and swine blood, as well as in horse blood. When the test serum was diluted 1 in 50, although turbidities formed in one of the above heterologous bloods, no flocculi appeared in two hours, except in horse blood, which gave a flocculent precipitate in thirty minutes. With dilutions of test serum 1 in 100 the heterologous bloods failed to react except by very faint turbidity, while in horse blood the reaction was but slightly diminished. Sheep precipitin from the rabbit failed to effect human blood in any dilution. Throughout their experiments the most distinct turbidities usually appeared in the more concentrated solutions of blood but usually the turbidities became flocculent first in the weaker solutions of blood.

The writers have tested various antisera with a view to determine under what conditions the reactions in heterologous bloods may be avoided, and without danger of error from the personal equation in judging the reaction. The action was first tested of normal rabbit serum, added in proportion of 1 in 1 to fresh serum of man, beef, sheep, goat, horse, rabbit, dog, cat, and chicken, diluted 1 in 10, 1 in 50, and 1 in 100. Faint turbidities were sometimes produced, but none of these were pronounced and none became flocculent until bacteria developed. Four humanized rabbit sera were then tested on the same bloods diluted from 1 in 10 to 1 in 500. The most active of these sera caused a flocculent precipitate in two hours, in human blood diluted 1 in 100 when added in the proportion of 1 in 200. The others were slightly less active. The results with these four sera varied considerably, some proving more specific than others. When diluted less than 1 in 30 all of the sera occasionally produced turbidities in heterologous bloods. These turbidities were usually slight and none of them obtained by dilution of test-serum 1 in 30 became flocculent in three hours, while all of them caused flocculent precipitates in three hours in human blood without regard to the dilution of this blood, when added in dilution 1 in 30 or even 1 in 100. It thus appeared that in order to obtain a specific reaction for human blood free from danger of error from the personal equation, one had to dilute these antisera at least 1 in 30, and require a distinct flocculent precipitate within three hours at 37° C. (See Table I.)

It was noted that among the animals tested, sheep, beef, and goat blood reacted more than the others, while horse, cat, rabbit, and dog bloods reacted very faintly, or not at all, and only with the earlier dilutions of the serum. The reactions with monkey blood will be considered later. The most active serum followed the rule of affecting most prominently the heterologous bloods but proved the most satisfactory to work with since it precipitated human serum in two hours when diluted 1 in 200, far beyond the dilution where heterologous bloods gave any response whatever. Even the lower monkey bloods, Java, rhoesus, baboon, failed to react with this serum diluted 1 in 100.

Next, a potent beef-rabbit serum was tested on the above specimens of blood serum with results similar to those reported by Kister and Wolff for horse-rabbit serum. This serum in dilutions of 1 in 5 caused flocculent precipitates in nearly all the bloods within three hours. In dilution of 1 in 10 (test-serum), beef, sheep, and goat blood, reacted alike. At dilution of 1 in 30 (test-serum) sheep blood failed to react, but a dilution of 1 in 50 (test-serum) was required before the goat blood gave no flocculi. At 1 in 100 (test-serum), the beef blood still gave a flocculent precipitate in two hours. (See Table II.)

Throughout the above series of tests, which numbered several thousand, it was rather uniformly observed that the flocculent precipitates formed most rapidly in dilutions of blood of about 1 in 100. It was frequently noted that in the tube of blood diluted 1 in 10 or 1 in 20, the turbidity failed to show any flocculent character at a time when in dilution of 1 in 100 the precipitate had fallen to the bottom, all the tubes having received the same amount of test serum. The most important factor in this result appears to be the inhibiting effect on the formation of flocculi exerted by the higher content of albumins. As the result was the same with different quantities of test serum it does not seem possible to refer it to different degrees of saturation of the precipitable molecule with precipitin, although this factor may be prominently concerned. Eisenberg refers it to a relative solubility of the precipitum in an excess of precipitable substance.

Finding that each test serum varied in its strength and specificity of action, it was thought possible that a precipitin prepared in an animal more distantly related might yield a more spe-

cific precipitin, since the scope of its cell-receptors might not include so many of those jointly shared by mammalia. Accordingly, a chicken was immunized by fifteen injections each of 10 c.c. defibrinated rabbit blood. Its serum proved very faintly hemolytic, but powerfully agglutinating for chicken blood cells, and caused precipitates in dilution 1 in 100. This serum had no effect on monkey and human blood in any dilution, but acted diffusely on the sera of several domestic animals. Another chicken received 7 injections of 10 c.c. of human placental blood. Its serum failed to affect the sera of domestic animals, or lower monkeys, in any dilution, 1 in 1 or higher, but produced an abundant flocculent precipitate in human blood 1 in 100, when added in a dilution of 1 in 50 (test-serum) in ninety minutes.

From the above experiences it seems possible to define the essential features of a specific reaction with the precipitin test. Regarding the *potency of the serum* it must be concluded that only the more active sera can safely be employed, such as cause flocculent precipitates in three hours when added in proportion of 1 of test serum to 100 of blood solution.

A potent serum is especially necessary when dealing with small blood stains. As the more potent sera are more diffuse in their action, and show certain individual variations in their effects on heterologous blood, it becomes advisable to test each serum on the blood of several domestic animals. The chicken may, provisionally, be recommended as an animal producing a more specific human precipitin than the rabbit. The *dilution of the test serum* must be high enough to rule out spurious reactions from heterologous bloods. It may safely be concluded that when test serum is added to dilute solutions of blood in the proportion of 1 of test serum to 50 of blood solution, only human and monkey bloods will give more than faint turbidities, and that a dilution of 1 in 50 is therefore a safe limit to prescribe. A dilution of 1 in 100 (test serum) may better be employed when the material to be tested is abundant, but with very small stains, yielding very weak solutions, this dilution of the test serum may fail to give a satisfactory reaction.

The *time limit* is a very important part of a specific precipitin reaction. Although Nuttall has found it advisable to measure the quantity of precipitin obtained after twenty-four hours, this lengthy period allows the access of several dis-

turbing factors, which may be avoided by a shorter time limit; principally the growth of bacteria which commonly develop in fluids which have not been passed through a Chamberland filter. Most writers who have employed the test for medicolegal work have preferred to read the reaction at the end of three hours in the thermostat, in which time the disturbing effects of bacterial growth are not apparent, and turbidities in heterologous bloods are less prominent. Moreover, the rapidity with which flocculi form in homologous blood is a very striking distinction from the slower changes occurring in heterologous blood. Hence the objects of medicolegal work seem to require a somewhat different procedure from that best suited to the exact measurement of blood relations among animal species. The formation in three hours of a flocculent precipitate visible to the naked eye, is a requirement which effectually escapes the influence of the personal equation and is the standard now generally employed in descriptions of a specific reaction.

The question still remains as to what reliance can be placed on less definite reactions, in which the turbidity does not become flocculent in six to twelve hours, and which may often be encountered when dealing with old and altered stains. Under such circumstances, one can only conclude that the significance of the results must be determined by all the circumstances in the case, including the age and quantity of the specimen, the strength of the turbidity, and the potency of the serum, and must remain a matter of opinion on the part of the operator.

In connection with the less definite reactions one may profitably remember that humanized sera diluted at 1 in 30 rarely produce prompt and distinct turbidities in any other than human and monkey bloods, and that even in dilutions of 1 in 10 or 1 in 20 (test serum) the turbidities in heterologous bloods seldom become flocculent in three to four hours. It is in such cases that control tests are of great value to determine just what reactions can be secured with heterologous bloods by the particular serum in use.

When, therefore, one has to deal with minute or old and feebly reacting specimens, it appears to be permissible to increase the time limit to four to five hours as recommended by Strube, and to increase the strength of the test serum to 1 in 30 or 1 in 20, but with each concession of this sort the results become less certain.

While convinced that the most reliable conclusions in medicolegal work are to be obtained by demanding, for the specific reaction, a flocculent precipitate in three to four hours, it must be admitted that the estimation of the bulk of precipitum may in some cases prove to be a necessary and trustworthy standard. In dealing with old specimens of human blood, the writers have found that the action of the serum is for some reason retarded and the precipitum is very slow in becoming flocculent, although at the end of twenty-four hours the bulk of the precipitum, as compared with the controls, leaves no doubt that the specimen is human blood. In such cases, if numerously and satisfactorily controlled, there appears to be no good reason for excluding the results obtained after twenty-four hours from those which can be regarded as specific. The controls in such cases should include a specimen of the tested fluid untreated by any serum, and one treated by some other antiserum.

The dilution of the tested fluid is a subordinate matter in the precipitin test. The writers believe that the data are sufficient to recommend a dilution of blood in the proportion of 1 in 100 as most favorable to the development of flocculent precipitates. Nuttall uses a solution of one part of blood to 20 of .6 per cent. salt in his method of estimation of the bulk of precipitum. Any accurate measurements in dissolving dried blood being impossible, we may employ Nuttall's expedient of shaking the fluid to determine if there is albumin in solution by the foam produced. Unless solutions are slightly colored reactions are very faint and flocculent precipitates cannot be expected, but blood pigments fade markedly with age. Corin obtained precipitates in solutions of blood which failed to give the spectroscopic reactions of Hb.

Unit Strength of Precipitin Sera.—It would greatly facilitate general agreement as to what constitutes a specific reaction if some means were devised of definitely determining and expressing in units the quantity of precipitin used in tests.

Von Dungern employed a rough method which can hardly be recommended. Wassermann and Schutze prepared a standard solution of blood as follows: 1 c.c. of fresh defibrinated blood was soaked up in linen cloth and dried several days or weeks. Two such specimens were then completely dissolved in 10 c.c. of .85-per-cent.

salt solution, filtered, and divided into equal portions. To these were added varying proportions of precipitin serum. They designated as a normal serum one of which the addition of 1 c.c. to 5 c.c. of the above standard solution exactly sufficed to yield a distinct flocculent precipitate in one hour at 37° C. If 1 c.c. of serum sufficed it was designated as a 10 N. serum. The amount of precipitin in 1 c.c. of N. serum they designate as a precipitin unit. There are several difficulties in the way of a satisfactory general employment of this unit, including the alterations in the drying of the blood, and the probable variations in the amount of albumen secured by dissolving the stain.

It is much safer to employ fresh plasma or serum for the standard solution, and a dilution of 1 in 100, since that strength seems to be the most favorable for the formation of flocculi, (cf. Eisenberg.) Hence the writers would suggest that a normal test serum be designated as one of which 1 c.c. added to 10 c.c. of fresh serum diluted 1 in 100 yields a distinct flocculent precipitate in one hour at 37° C. and that the unit of precipitin be the quantity contained in 1 c.c. of such normal serum. The average serum obtained after 6 to 8 injections of 10 c.c. of blood in the rabbit would then be of 10 N. strength and 1 c.c. would contain about 10 precipitin units.

On the Differentiation of Monkey Blood.—Stern first noted the fact that monkey blood reacts to humanized rabbit serum much more distinctly than that of any other lower animal. He tested three species, *Cercopithecus*, *Macacus cynomolgus*, and the "Kronen-affe." Nuttall tested the blood of 46 monkeys and apes finding that the *Simiidæ* yielded almost as much precipitum as man, the *Cercopithecidæ* yield less, while the *Hapalidæ* and *Cebidæ* give but little. He observed that weak humanized sera affect only the two first named groups, while a powerful serum will include them all. Nuttall and Strangeways compared the bulk of precipita forming in human and some monkey bloods with the following results. Human serum 100 per cent., *Macacus* 90 per cent., orang 80 per cent., *Cynocephalus* 70 per cent., mandril 50 per cent., *Cercopithecus* 50 per cent. The first three monkeys named died of dysentery or intussusception and it was suggested that the manner of death might have altered the precipitable substance of the blood.

One of us has attempted the differentiation of monkey and human blood by calibrated serum,

but the work is still incomplete and will be presented at another time.

Up to the present stage of this work, it has proven possible to distinguish between human and lower monkey bloods, Java *Ineues cynomolgus*, *rhæsus*, *Macacus rhæsus*, baboon, *Cynocephalus ursinus*, by diluting humanized serum 1 in 100, with which strength the blood sera of the above monkeys failed to yield a flocculent coagulum in three hours, and in fact gave but slight turbidity in that time. This rabbit serum, however, failed entirely to separate the blood of the chimpanzee, *Simia troglodytes*, and orang, *Simia satyrus*, which reacted as strongly as did human blood. On the other hand, a humanized chicken serum failed in any dilution, to affect the sera of the above lower monkeys, and in dilution of 1 in 100 chimpanzee blood became slightly turbid, but failed to yield a flocculent precipitate in several hours, although the human blood treated by the same diluted serum gave a flocculent precipitate in one hour. It thus appears that by careful calibration of the test serum the blood of the highest anthropoid monkeys may be distinguished from human blood. In this work it seems to be essential that the test serum be fresh and the bloods tested be fresh, or of about equal age if obtained dry or from the cadaver.

Layton has found that the blood of certain monkeys, variety not stated, may be distinguished from human blood by diluting the blood to 1 in 600, and adding the serum undiluted in the proportion of about 1 in 60.

TECHNICS.

The Production of Precipitin Serum.—*I. Animals.*—The rabbit is the animal generally used for the production of precipitin serum. It is easily handled, and reacts to almost every type of blood. Rabbits vary very much in their reaction to injected blood. Some produce very strong antiserum, others very weak, and still others, almost none at all. The fact that rabbits have individual idiosyncrasies in this respect has been noted by several observers. We also found that the common rabbit very often could not withstand the toxic effect of the injection and emaciated very rapidly, some of them succumbing to the treatment. Besides rabbits we used full-grown Belgian hares and crosses between them and rabbits, and they proved not only very resistent to the injections, but also yielded

our most potent sera. Too much stress cannot be laid upon the care bestowed upon the animals under treatment. They are often sick from the injections, so that they require plenty of food and clean, airy quarters. It was soon found that the healthier the animals the better were the results with the sera.

Corin and Biondi, Arthus and Vansteenburgh, and Hausner, used dogs in order to get larger quantities of serum and they report satisfactory results. Uhlenhuth injected a lamb and a goat with defibrinated human blood but did not obtain a good serum. One of our most potent sera was obtained from a hen which had received intraperitoneal injections of defibrinated blood. The bird gives very little evidence of any reaction to the injection, and being further removed, biologically, from man, than the rabbit, ought theoretically to yield more selective antisera. Wassermann and Schutze have been testing the serum of a goose but have not yet published their results.

II. Choice and Preparation of Material for Injection.—In order to produce an antiserum for human blood, either defibrinated human blood, blood serum, or some fluid derived from the body, and containing globulin similar to that of normal human blood serum, must be injected. Therefore exudates and transudates, such as can be obtained in the pleural sac and abdominal cavity, which contain a certain amount of serum globulin, are suitable substances for injection.

Defibrinated human blood has generally been used. We obtained it either from the umbilical cord or by squeezing the placenta. The blood from either of these sources was received into a sterile Erlenmeyer flask containing glass beads the size of small peas. If beads are not obtainable, sand, or even broken bits of glass, which have been thoroughly cleansed and sterilized, may be used. Vigorous rotation of these flasks for five minutes sufficed to defibrinate. Some investigators use only the serum free from cells. While it makes little difference with the strength of the antiserum obtained, since only the serum globulin is the active agent in the process, we found it more convenient and less troublesome to use defibrinated blood rather than pure serum. Wassermann and Schutze and Nuttall use serum; Uhlenhuth uses defibrinated blood. Uhlenhuth obtains his blood fresh from the subject by means of Heurteloup's cups (a kind of wet cup). It

could easily be obtained from the veins by Thacher's apparatus.

Uhlenhuth found that old dried blood dissolved in .6-per-cent. salt solution yielded an active precipitin serum. Grigorjew used dried blood, prepared as follows: 10 c.c. of either human serum or whole blood is poured on a large glass plate in a dust-free room, at ordinary temperature. The pure blood serum dries in from four to six hours, that mixed with blood in from two to four hours. To prepare this dried serum for injection, he pours 10 c.c. of physiological salt solution over the plate, scrapes the serum or blood loose and stirs it until a uniform solution is obtained.

In order to prove that the drying of the blood did not have any influence upon the formation of precipitin, Grigorjew divided into six portions 3.6 grm. dried calf's blood, three years old, dissolved each portion in 10 c.c. of 1.6-per-cent. salt solution, and injected it subcutaneously into a rabbit. The serum of the rabbit gave a precipitin reaction with calf or beef blood spots. Grigorjew's method and experiment are important because fresh human blood is not always obtainable, and in such a case, a supply of dried blood would prove of much service.

Ziemke has made use of blood from the cadaver in cases where death was due to hemorrhage. He has taken it from cadavers which were three and four days old and even older and none of his rabbits became infected. His method is to place the blood in high glass cylinders, pipette off the serum into sterile Erlenmeyer flasks and add chloroform in the proportion of one part to ten of serum. If he had doubts as to the sterility of the serum thus prepared he injected it subcutaneously rather than intraperitoneally. His results were almost, though not quite, as good as when fresh blood was used. Minovici found that cadaver blood, even when fresh, gave a weaker serum than placental blood. Minovici injected amniotic fluid and Ziemke tried hydrocele fluid. Both found that the precipitin serum obtained was weaker than that obtained with human blood or serum. The same holds true when ascitic fluids or pleural exudate are used, since as Biondi has pointed out, they contain only one-third the amount of serum globulin contained in blood. Hence larger quantities and more frequent injections are required. With pleural exudates especially it is found that the animal may not withstand the toxic substances often contained in such

fluids, and the danger of infection is also greater. Albuminous urine has likewise been employed. Dieudonné used a urine which registered 3 to 7 grm. of albumin per thousand (Esbach), Le-Clainche and Vallée, a urine containing 1-2 grm.; Mertens one containing 4 per cent. albumin; and Zuelzer a 1 to 9 per cent. specimen. The animals usually suffered considerably, many injections were required at long intervals, and the resulting serum was not extremely active.

Corin has devised a method for utilizing ascitic fluid which yielded very satisfactory results. He saturates ascitic fluid with magnesium sulphate at a temperature of about 30° C. The resulting precipitate is dissolved in water and this solution again saturated with the sulphate. This precipitate is then allowed to dry on the filter. For injection, it is only necessary to dissolve the dried precipitate in water, as the magnesium sulphate is not harmful to the animal. Corin estimates that the solution thus obtained contains 4.5 per cent. paraglobulin. By this method a concentrated solution of globulin can be injected at frequent intervals. Ammonium sulphate cannot be used because it throws down albumin as well as paraglobulin; because the precipitate does not dissolve easily in water; and the solution when obtained is found to cause a cloudiness in human serum through the action of the salt.

In the present work it was found that either fresh serum or defibrinated blood yielded the best results. Our results with other albuminous fluids were unsatisfactory, and we do not believe that any other material can safely replace fresh whole blood, when medicolegal work is proposed and active selective sera are required. Most of the human blood was obtained from the cord and generally remained sterile. The quantity obtained at one time was necessarily small, amounting on the average to two ounces. By squeezing the placenta, larger quantities of blood can be obtained, but there is more likelihood of contamination. Another factor, which seems to have some bearing on the production of a potent serum, was the freshness of the blood. The reaction of the rabbit after injection of a fresh specimen was always more pronounced and more lasting than from specimens which had been kept in the refrigerator a few days. Uhlenhuth and Minovici noted this same fact. Beef, sheep and other bloods obtained from the slaughter-house should

be tested bacteriologically before using, since they are frequently contaminated with vomitus.

III. Methods of Injection.—The injection may be either subcutaneous, intraperitoneal or intravenous. Each method has its adherents, and produces an equally good antiserum when the other conditions are equal. Uhlenhuth, Biondi, Corin, Nuttall and Minovici, injected intraperitoneally. Grigorjew, Dieudonné, Arthus and Vansteenburgh, Okamoto, Stern, and Wassermann and Schutze, injected subcutaneously. Graham-Smith and Sanger, LeClainche et Vallée, Mertens, and Strube, prefer intravenous injections. The technic of subcutaneous and intravenous injections, requires no special comment. The method of intraperitoneal injection is more complex and requires greater care. The method employed by Minovici is as follows: Animal held head downward, in order to get the internal organs out of the way, hair cut away with scissors from lower part of abdomen and skin sterilized with 2-per-cent. sublimate solution; epidermis cut with sterile scissors, and needle pushed through the wound into the abdominal cavity; wound closed with cotton and collodion. He injected twenty rabbits by this method and had no infection.

Nuttall shaves the abdomen, sterilizes the skin with lysol, and punctures the skin in the lower left hand region of the abdomen with a scalpel. Then he bores through the fascia and the muscles with a blunted hypodermic needle and afterward applies tr. benzoin to the cut.

Biondi draws up the blood or serum into a long sterile glass tube which has been drawn out to a point at one end. The other end is closed with sterile cotton. He disinfects the skin of the abdomen with 10 per cent. sublimate and then bores through into the peritoneal cavity with the capillary end of the tube.

The method we employ is as follows: An area about the size of a half dollar, just below the middle of the abdomen, is shaved and disinfected either with lysol or sublimate solution. An assistant holds the rabbit by the ears with one hand and places the other behind the animal's shoulders keeping the head stretched well backward. In this way the greater part of the body hangs free, and the skin of the abdomen is tense. The needle is then inserted at any point in the part prepared. It should be inserted through skin and muscle slowly and with steady pressure and as soon as it

enters the peritoneal cavity, which it does with a jerk due to the absence of resistance, it should be turned in such a manner as to be along, and close to, the peritoneum covering the abdominal wall it has just entered. The needle may then safely be inserted a sufficient distance to ensure its remaining in the body while the blood is injected. The object of turning the needle as soon as it is through the peritoneum is to avoid puncture of the gut which is the only danger in the method. By practice one soon learns to feel the jerk which signals the entrance into the body cavity. If the needle is sharp and is inserted slowly and with even pressure the rabbit will remain perfectly quiet when held as directed. Should the rabbit be refractory, it will be necessary to hold the hind legs extended. In so doing the animal's back should be arched so as to cause the abdomen to bulge. The object of this is to prevent the needle merely passing through the skin and subcutaneous fascia and pushing the muscle wall in front of it, so that a subcutaneous injection results instead of an intraperitoneal. The needle should be of fairly large caliber, a hypodermic is too small, and should have a length of at least two inches. If the needle is so large that it leaves a canal through which the blood oozes from the peritoneal cavity, a valve can be made to close it by first drawing the skin to one side and puncturing, after which it is allowed to retract into place and thus cover the hole through the fascia and muscle wall. After puncture, no collodion or other dressing is necessary.

IV. Number and Frequency of Injections.— The number and frequency of injections required depends on which route is employed, intraperitoneal, subcutaneous or intravenous. Strube states that on an average six intravenous injections of 1 c.c. of defibrinated blood given in the course of eight days, produce a serum yielding a positive reaction in human serum diluted 1 in 1,000. He admits that much depends upon the individual peculiarities of the rabbits. In one instance he obtained a serum which gave a reaction in 1 in 20,000 after injections covering a period of fourteen days. He does not state how much or what blood or how many injections he used. Mertens at first injected subcutaneously and then changed to the intravenous method. He injected urine containing albumin every day for seven days to the amount of 35 c.c. LeClainche and

Vallée also injected urine intravenously, 20 c.c. at a time, and a total of 150 to 200 c.c.

The latest investigators using the intravenous method are Graham-Smith and Sanger. They injected about 18 c.c. divided into portions of 5, 5, 5, and 3 c.c. at intervals of two, three, and four days. They found this to be sufficient to produce a powerful antihuman serum. Powerful antibeef and antisheep sera were obtained after the intravenous injection of 9 and 12 c.c., in doses of 1, 2, 1, 3, and 2 c.c. and 1, 5, 2, and 2 c.c. or 3, 5 and 3 c.c., respectively. The intervals between the injections were 4, 4, 2 and 5 days in each case. We are in no position to criticise this method, having used only the intraperitoneal route, but suggest that more uniformity is desirable. Some investigators, among whom is Uhlenhuth, report poor results with the intravenous method, and prefer one of the other means. It is also difficult to form any idea of the strength of the sera thus obtained, because the descriptions of the reactions are incomplete.

Wassermann and Schutze are the most prominent investigators to use subcutaneous injections. They obtained their sera by five or six injections of 8 to 10 c.c. of serum, every second day. This they claim gives a serum which is powerful enough to differentiate human blood. Grigorjew and Dieudonné injected 10 c.c. subcutaneously every third or fourth day, and used six injections. Arthus and Vansteenburgh employed five injections of 5 c.c. each, every fifth day.

Stern injects 5 to 10 c.c. beneath the skin at intervals of two days or more, depending upon the condition of the animal. Okamoto also used the subcutaneous method. He injected amounts of blood varying from 12 to 15 c.c. at irregular intervals, which he determined by fluctuation in the rabbit's weight. Total quantity injected was from 60 to 85 c.c.

The intraperitoneal injection, which is the method generally used, is employed by Uhlenhuth. He injects 10 to 20 c.c. at intervals of six to eight days, until he has injected a total of 60 to 80 c.c. Ziemke injects every third to fourth day, depending upon the behavior of the animal's weight, until the total of 60 to 100 c.c. is reached. Minovici used six to eight injections of 10 c.c. each, of fresh blood, every fifth or sixth day; older blood every second day. Honl injects 5 to 10 c.c. every third or fourth day. Nuttall, whose work in this field covers the most ground,

injects intraperitoneally, five or six times. He commences with 5 c.c., and then, after the third injection, increases the quantity to 10 c.c. He regulates the interval between injections by waiting until the animal regains the weight lost after a previous injection. Biondi injects intraperitoneally 5 to 10 c.c., at intervals of two to four days, until the rabbit has received from 50 to 80 c.c. He obtained his best serum from the rabbit which had received 40 c.c. within 24 days.

Our experience has led us to prefer the intraperitoneal injection, because of the ease of execution, the small chance of infection when a little care is exercised, the fact that rabbits react promptly when foreign matter is introduced into the peritoneal cavity, and because the peritoneum is tolerant to such injections. We have found that the most suitable amount for the single injection of human defibrinated blood or serum is, as a rule, 10 c.c. If the blood or serum is fresh, it is safer to inject at first only 5 c.c. This is especially true of the serum of certain horned animals, as beef, and sheep, which are more toxic to the rabbit than human blood. We have injected as high as 20 and 30 c.c. of serum at one time, but these large quantities did not increase the potency of the serum. On the contrary, they seemed to inhibit the formation of antibodies.

We found that injections at an interval of four days, yielded the best results. Some observers use the weight of the animal as a guide to the repetition of the injection. We did not find it necessary to resort to this measure, because one sees at a glance whether or not the rabbit is doing well, if it feeds normally and is as active as it should be. Those who observe the weight of the injected rabbits also find that a three or four day interval is the most suitable. If the blood is old or if the rabbit is very tolerant, injections may be made as often as every two days. But not much time is gained in such cases, since it would appear that the animal requires a larger number of injections to develop a powerful antiserum. When a rabbit does not appear to be doing well, treatment should be omitted, until it has recovered. Nothing is gained by injection at such a time, because it is almost certain to cause further emaciation and is just as certain not to cause the formation of a good antiserum. The number of injections required we found to vary, according to the material used and the idiosyncrasy of the rabbit, from five to eight; the total

quantity of serum being from 50 to 80 c.c. This experience accords with the observations of Uhlenhuth, Wassermann and Schutze, Biondi, Nuttall and others. We found that if after eight injections, a satisfactory serum was not obtained, further treatment was useless, as the animal was apparently incapable of yielding a good serum. On one occasion Schutze, in this laboratory, obtained a very active precipitin after two injections of a total of 20 c.c. of defibrinated blood. With subsequent injections to the total number of seven, there was no improvement, in fact the serum became weaker. Nuttall noticed that some rabbits yielded a good serum after the third injection. If injections are continued after an animal has developed an active antiserum, the strength of the serum may be increased, but sometimes it is diminished, as observed by Uhlenhuth and ourselves. In one instance an animal giving a serum which yielded flocculent precipitates in one hour when added to human blood (1 in 100) in dilution of 1 in 200, received two injections in four days of 20 to 30 c.c. of fresh blood. One week later its serum diluted 1 in 50, failed to precipitate human blood 1 in 100 in three hours. At the same time the animal began to emaciate. Stern was able by repeated injections and with due regard to the condition of the rabbits, to produce an antiserum which reacted in blood 1 : 50,000.

It sometimes happens that after a rabbit has received eight injections, it yields an antiserum which is not as powerful as desired. If, now, this rabbit receives no further treatment for at least one month and then injections are renewed, the potency of the precipitin may be found to have increased. One of our antibeef rabits did not yield a good antiserum after several large injections. It had not lost appreciably in general condition. Nothing further was done to the rabbit for three weeks, when the injections were resumed. It then yielded a much more powerful antiserum than before. Strube reports that one rabbit, which had received 8 c.c. of blood intraperitoneally in January and had a value of 1 : 5,000, was reinjected in April, with the same quantity of blood, and gave a reaction in 1 : 20,000 dilution of human blood.

Uhlenhuth first called attention to the fact that some sera from immune rabbits have a milky, opalescent appearance. He noticed that this opalescence increased after intravenous injections. Physiologists ascribe its occurrence to digestion.

Nuttall noticed it and thinks it due to very large injections. He also remarked that some of the rabbits yielding this serum suffered from cysticerci of the liver. Uhlenhuth and Rostocki have shown that it cannot be removed by filtration through porcelain. We noticed it in one case which had received large injections. Such sera are untrustworthy and should not be used.

The *course of the development of blood precipitins* has been followed by v. Dungern who describes four periods: (1) a *latent period* following the injection, during which the blood fails to show any precipitin, (2) a *period of increase* of precipitin, (3) a *period of equilibrium*, when the precipitin does not vary, and (4) a *period of decrease* of precipitin. In fresh animals a period of four to six days followed the first subcutaneous injection of foreign plasma before any precipitin appeared in the blood. The precipitin then increased in quantity reaching its highest development in about two days. A short period of equilibrium then followed or the decrease began at once and continued irregularly, with considerable variations in different animals. If a new injection is given, at the time when the precipitin has reached its highest point, within one-half hour most of the antibody disappears, being saturated by the new plasma, and the latent period before the reappearance of precipitin is shorter than after the first injection and continues to shorten after succeeding injections. After successive injections the increase of precipitin is usually much greater than after single doses and persists longer. Five minutes after single injections the foreign albumin was demonstrable in the blood by the precipitin test, but disappeared within one hour. After successive injections the disappearance of the foreign albumin was slower.

V. Methods of Bleeding.—The progress of development of precipitin in the blood, may be determined from time to time by drawing a little blood from the lateral ear vein of the rabbit. These preliminary tests of the serum should not be made less than three or four days after an injection, since each injection of blood causes a temporary diminution in the precipitin. The ear is shaved, sterilized with lysol, sublimate or other disinfectant, and washed with sterile water. It is well to suspend the rabbit by the hind legs, and to have an assistant gently compress the vein at the base of the ear. When the vein is cleanly cut the blood flows to the tip of the ear and can

be caught so that it drops to the bottom of the test-tube. When there is about 5 c.c. in the test-tube it is laid in a slanting position, permitted to clot, and then placed in an ice chest. If it is found that the serum does not exude from the clot, the latter should be loosed from the test-tube by means of a platinum loop. The serum will also be found to collect better when the tube is out of the ice chest for a time.

There are three practical methods of obtaining large quantities of serum from the rabbit. The first is by bleeding from the ear veins. We have already described the procedure. As much as 40 c.c. of blood may be had at a single bleeding and if care is exercised the blood obtained can be kept sterile.

Wadsworth (1903) demonstrated, before the New York Pathological Society, a method whereby the blood is made to flow profusely from the lateral ear vein. The rabbit is held head-downward and wrapped in a blanket. Inside the blanket and next to the spine is placed a hot water bag with the result that the peripheral vessels are dilated by overheating. The second method of bleeding the rabbit is one which entails the death of the animal, and consists in bleeding from the heart. As practised by Uhlenhuth, the procedure is as follows: The hair is removed from the chest-wall, the skin sterilized, and the anterior chest-wall cut away with sterile scissors. The left ventricle is then cut and the blood allowed to flow into the pleural cavity, whence it is sucked up with a sterile pipette, put into flat dishes or cylinders, allowed to clot, and the serum removed later. Minovici modifies this method in that the pulsating heart is lifted out of the chest and held in the mouth of a vessel into which the blood flows when the ventricle is cut. The third method is that of bleeding from the carotid artery. It is frequently used, because a large quantity of blood is obtained without sacrificing the animal. The rabbit is fastened securely on its back, the head well extended and the throat shaved. The skin is then disinfected and an incision is made in the median line, extending from above the larynx to the sternum, and through the skin and fascia. Then by means of blunt dissection the muscles along the trachea are separated and the carotid exposed. The vessel must be freed from the pneumogastric nerve, which lies in the same sheath with it and in doing this care must be exercised to avoid tearing the vessel. When the caro-

tid is isolated it is clamped as high up as the incision will allow and also as low down as possible. The vessel is then divided near the upper clamp and seized securely by its fascia, with small pointed forceps near the cut end. The lower clamp is then loosened and the stream of blood can be directed into the receiving vessel or into test tubes which are afterward laid in a slanting position. When a test tube contains enough blood the artery clamp can be temporarily closed until the next is ready. Sometimes a clot forms at the mouth of the vessel and stops the bleeding. This is easily removed by stripping the vessel with a pair of anatomical forceps. When sufficient blood is obtained, both ends of the carotid are tied off and the wound sewed. If it is desired to exsanguinate the animal, it will be found advantageous toward the end to firmly compress the body with the hand, commencing at the lower end of the abdomen and ending at the chest. In this way almost every drop of blood can be squeezed out of the body. Some investigators insert small glass tubes into the carotid but this is unnecessary and complicates the operation.

In bleeding a bird the blood is taken from the large vein on the under surface of the wing, which is plainly seen beneath the skin at the angle between the wing and trunk. It bleeds very freely.

A considerable interval should elapse after the last injection, before the blood is removed for use in tests. From experiments conducted principally on the development of bacterial precipitins, it has been found that the greatest development of immune bodies is seen from one to two weeks after the last of a series of injections, and the same rule has been found to apply in the development of blood precipitins. Some observers regard six days as sufficient, while others allow fourteen. We have always obtained active sera after an interval of seven to ten days.

There are at hand but few definite observations regarding the *persistence of active precipitins* in the blood of animals employed. Strube found in eight rabbits which had been treated intravenously that the sera retained a value of 1 in 1,000 for four weeks. Later they diminished rapidly in strength, for after eight weeks three animals which showed original strengths of 1 in 5,000, 1 in 1,000, and 1 in 1,000, had almost completely lost their activity. Graham-Smith and Sanger found that some sera retained their specific quali-

ties for twelve months, while others deteriorated more rapidly. Nuttall noted that some sera became untrustworthy after a time, giving cloudings with all sera.

Our chicken immunized to human blood, and whose serum originally gave a flocculent precipitate in one-half hour in human serum 1 in 100, when added in dilution of 1 in 50, very rapidly lost its potency and was practically inert four weeks after the last injection.

Of two humanized rabbits receiving nine injections and then yielding a serum which at dilution 1 in 30 gave a flocculent precipitate in one hour, we found that one continued to yield a powerful serum for at least twenty-three days (flocculent precipitate at 1 in 30 in one hour) but the other had lost much of its activity in that time (moderate turbidity at 1 in 30 in three hours). A beef rabbit, also, after twenty-eight days, gave a serum of undiminished activity, while a humanized rabbit strongly immunized but emaciating from ringworm, gave an inert serum after eight months. Comparing these observations with those on the changes in serum stored on ice, it appears that the specific precipitins are much longer retained in the test tube than in the animal's body, but that a strongly immune animal may be expected to yield a good serum for at least three weeks after the last injection.

Methods of Applying the Precipitin Test. —Many of the details of the application of the precipitin test have been fully considered in the theoretical discussions of this article. Other matters which deserve mention, concern the methods of dissolving the suspected stain, its clarification, the instruments to be used, and the scope of necessary controls. The solution of the stain should be made in .6 or .8 per cent. of NaCl (c. p.). A fresh stain dissolves promptly, but very old stains may require soaking for some hours. Ziemke has recommended .1 per cent. soda solution as more active than salt in dissolving old stains, and he reports some positive results obtained from such solutions when the solution in salt was negative. That some of the reactions were referable to the soda and not to a specific reaction on the albumins is suggested by the observations of Graham-Smith and Sanger, who obtained some spontaneous precipitates when blood serum was dissolved in .1 per cent, caustic soda. The use of this solvent cannot at present be endorsed. Ziemke also claims to have secured

positive results from stains entirely insoluble in salt or soda by dissolving in concentrated solution of potassium cyanide and neutralizing by tartaric acid in substance almost but not quite to the point of neutralization to litmus. This procedure also introduces sources of error that are difficult to control, especially the abnormal content in chemicals. Graham-Smith and Sanger found that test serum is clouded in one hour by potassium cyanide 1 per cent., or tartaric acid 1 in 1,000, although the prompt neutralization of the acid prevented any precipitation of albumins. We have employed this method successfully, with proper controls, on comparatively fresh blood clots, but failed with an old specimen of blood in earth. It can be undertaken only with comparatively large stains. Schutze, in this laboratory, could not convince himself that the results with this solvent were trustworthy.

All solutions of blood stains from miscellaneous materials should be tested for their reaction to litmus, and if found distinctly acid or alkaline, should be neutralized by sodium carbonate or tartaric acid. If spontaneous or spurious reactions occur in solutions of blood from earths containing lime salts, these must be removed by a current of Co_2, and filtered (Graham-Smith and Sanger).

There appears to be at hand no means of removing from solutions of stains on leather the tannin which seems to be the cause of spontaneous and spurious reactions. The general topic of the removal from solutions of blood stains these and other disturbing agents which will doubtless be encountered from time to time offers a large field for further investigation.

Great care is necessary in the choice of chemicals used in serum work. The writers encountered a sample of NaCl guaranteed c. p., which caused spontaneous precipitates in various sera.

Filtration.—Most solutions of blood stains require filtration, since it is essential that the fluids be perfectly clear before adding the test serum. Filtration through Schleicher's paper (blue label) will clarify the great majority of solutions.

An asbestos wool filter backed by a perforated platinum scale is also very effective. An electric centrifuge is of much value as a means of clarifying many solutions.

Solutions of materials containing many bacteria should be passed through a small Berkefeld or Chamberland filter. Small test tubes, inside

diameter not less than one-half centimeter, are required, and a stock of sterilized pipettes are needed to handle the solutions and test serum.

Greenbaum and others, instead of using test tubes, have followed the reaction under the microscope in specimens prepared by adding one or more drops of test serum to a small quantity of solution on a glass slide or in a hanging drop. The use of alkali is in the cleansing of glass ware must be avoided.

Controls.—Without the employment of several controls, no reliable conclusions can be obtained from the application of the precipitin test.

The frequent occurrence of spontaneous precipitates demands that one specimen of the tested solution be carried through without the addition of any serum.

Since the solution may contain some agent slowly precipitating foreign albumins, it is necessary to carry through a specimen of the tested solution which receives the same quantity of some other antiserum or foreign serum as is used of the specific test serum. In testing for human blood the writers employed for this control a potent beef-rabbit serum, but normal rabbit serum may perhaps suffice.

Very welcome corroborative evidence is usually obtained when one carries through a known specimen of human blood of appropriate dilution to which is added the usual quantity of test serum.

As there is considerable variation in the specificity of different rabbit sera, careful workers prefer to test each serum on the blood of several domestic animals as well as on human blood, to determine not only the strength of the serum, but also its effect on heterologous blood. This control is the more necessary with the more powerful sera.

If the observation extends longer than three hours, the precipitum should be examined for the presence of bacteria, the growth of which is not always inhibited by chloroform.

Since any albumin from the human body may respond to humanized antiserum, it must first be determined by the hemin or spectroscopic tests that the material is blood. While it is possible that the blood of a domestic animal may be mixed with human albuminous exudates as sputum, pus, urine, or feces, such a combination must be exceedingly rare. A microscopical examination will usually determine the presence of mucus in sputum, excess of leucocytes in pus, and fecal de-

tritus. The gross appearance of the stain will usually determine the possibility of mixture with urine, or the demonstration of characteristic crystals of nitrate of urea may be undertaken.

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THE TOXICITY OF TETRA-PHOSPHORUS TRI-SULFID.* — A PRELIMINARY REPORT.

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Although phosphorus was introduced into manufactures early in the nineteenth century, and not long afterward began to be used against vermin in the well-known form of paste, it was not until about 1845 that cases of fatal poisoning by phosphorus in the human subject began to be collected. Since that time it has become a frequent means of suicide, especially among women and on the continent of Europe. The usual methods of taking phosphorus to produce death are the ingestion of match heads or of an infusion made from them, and swallowing a certain amount of phosphorus paste in some fluid vehicle.

According to Smita,[52] the average phosphorus content of a match head varies from 0.00178 gram to 0.000167 gram, but most commonly the variation is less, running from one-half to one milligram. If, therefore, a person swallows the heads of one hundred and eighty matches, or of two hundred and fifty matches, as in two cases of Krug's,[26] the actual dose of phosphorus may be as high as 0.09 to 0.125 gram.

The lethal dose of phosphorus is given by Kolisko[24] as ten to twenty centigrams, but Sonnenschein (cited in 24) reported a case of a child aged five weeks who sucked the head of a single match with fatal result. Vetter[59] gives the percentage of phosphorus in the usual samples of paste as from eight to fifty per cent, but as it is almost impossible to determine

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the amount of the paste taken, the actual dose cannot be accurately known.

With a view to ascertain the comparative toxicity of phosphorus tri-sulfid (P_4S_3), which has been employed in the arts within the last decade, the following investigation was begun. The literature of phosphorus poisoning was examined and abstracted and several experiments upon animals were made with P_4S_3. But little experimental work has been done upon this sulfur compound of phosphorus, the most important papers being those of Courtois-Suffit and of Sevène and Blanc. The latter observers found no marked effects in guinea-pigs which received 0.03 gram of phosphorus tri-sulfid in repeated doses, though 0.003 gram of phosphorus was uniformly fatal. Their results will be noted in detail below.

Tetra-phosphorus tri-sulfid was given to two dogs in moderate morning and evening doses for a period of eight days, and, after a pause of one week, for a second period of eight days in the same manner. To a third dog single large doses were given daily for four days. A fourth dog received a single extremely large dose. In the sixth experiment a rabbit was exposed under a bell jar to a moist atmosphere which had been passed through several layers of the compound.

Dogs I. and II. received a weighed amount of food; the metabolism of fat, nitrogen, sulfur, and phosphorus was charted; the dogs were killed and immediately sectioned, and the organs were hardened in alcohol, formalin, or Orth's fluid, and examined after imbedding in celloidin or paraffin.

HISTORICAL.

In order to bring out the contrast between the effects of phosphorus and those of P_4S_3, it will be well to give a summary of the main facts in regard to poisoning by the former, the chief clinical symptoms and the various lesions found, and then discuss the observations made during this investigation.

As is well known, the poisonous form of phosphorus is

the yellow, which is soluble in various media; the red form is non-soluble and non-toxic.

In the majority of cases of suicide, or murder, or accidental death by phosphorus, the onset and duration of the symptoms may be termed acute or sub-acute. The prominent symptoms are given by Lewin[30] as:

Severe gastric or abdominal pain in	92.0 per cent.
Vomiting	81.2 " "
Ecchymoses and hemorrhages	45.0 " "
Icterus	34.1 " "
Diarrhea	30.0 " "

Other symptoms which are often encountered are dyspnea, convulsions, coma, paralysis, suppression of urine, fever, severe thirst, clay-colored stools, abortion, etc. Hamilton and Godkin[18] state that ten to fifteen per cent of all cases die within twenty-four hours, and various observers report death within a few hours. Many cases, however, last for several days, and there may be an interval of comparative comfort after the first sudden symptoms before the case again becomes serious.

Taking the acute and the sub-acute cases together, death may occur in seven hours (Maschka), or as late as the seventeenth day (Hessler). The following partial table gives the variation in the time of death after taking the poison, with some other facts:

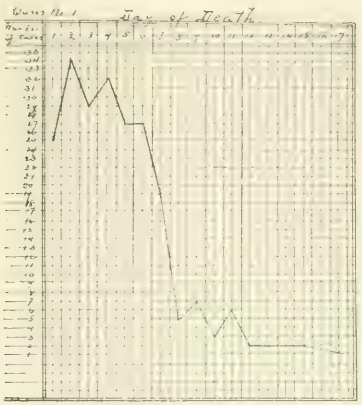
Observer.	Sex.	Age.	Form and Amount.	Day of Death.
1. Maschka	Child.	3 wks.	3 match heads.	Seven hours.
2. Hingel	F.	25 yrs.	Not given.	A few "
3. Kaustmann	F.	56 "	" "	24 "
4. "	F.	32 "	" "	30 "
5. Coupland	F.	14 mos.	Sucked matches.	44 "
6. Heberda	F.	21 yrs.	Not given.	3 days.
7. Maschka	F.	16 "	Match heads.	3 "
8. Burge	F.	15 "	100 match heads.	4 "
9. Pastau	F.	34 "	8 pkg. match heads.	5 "
10. Stumpf	F.	17 "	Not given.	5 "
11. Mialhe	F.	12 "	" "	5 "
12. Grose	F.	5 "	Paste.	5 "
13. Vollbracht	F.	?	2 pkg. matches.	5 "
14. Krug	F.	15 "	180 match heads.	6 "
15. "	F.	15 "	250 " "	6 "
16. Leonpacher	F.	78 "	Not more than 0.06	7 "
17. "	F.	17 "	Not given.	8 "
18. Oppolzer	F.	24 "	6 pkg. matches.	8 "
19. Buchner	M.	Adult.	Rat poison.	9 "

Of two hundred and nineteen cases collected from the literature, one hundred and ninety-three, or 88.12 per cent, died within the first seven days, as will appear from the annexed curve. Of two hundred and eight fatal cases cited in Witthaus and Becker,[4] the shortest duration was seven hours, the longest was fifty-three days, and the average five and one-quarter days. The percentage of fatal cases is there estimated at about eighty per cent.

The effects of phosphorus in the organism may be considered with reference to the human subject or to animals,

both subjectively and objectively during life, and in the lesions found post-mortem.

In from ten to fifteen per cent of the cases the onset of symptoms is rapid, but in the majority they appear only



Curve showing day of death in 219 cases of phosphorus poisoning: 193, or 88.12 per cent, died within the first seven days.

after several hours or days. There is a peculiar odor in the breath, there are nausea or vomiting, thirst, slight fever, diarrhea, and the vomitus and the feces may smell of phosphorus and glow in the dark. The feces may be clay colored or acholic, but as the color of normal feces depends upon the presence in the intestine of the products of both the liver and the pancreas, bile may be present even in seemingly acholic feces, if there be a lack of pancreatic juice in the intestine. The liver becomes large and tender. Epigastric and abdominal pain may be severe and continuous or intermittent. Icterus is common by the third day if the case live till then. Fever is seldom high, and toward the end the temperature may be subnormal. The pulse is usually feeble, and this may progress to complete cardiac

failure, as might be expected from the fatty changes in the heart. Delirium and convulsions are not uncommon, and coma, if present, may resemble the uremic form.[57]

The urine is frequently either diminished or suppressed. Bettelheim[3] found a great increase in the alkaline phosphates in the urine. Free fat, albumin, blood cells, bile pigment, and both leucocytes and epithelia in fatty degeneration have been found in the urine. Sarcolactic acid,[18, 20] leucin, and tyrosin occur in the urine, and Bauer found paralactic acid. The urea is at first diminished, then increased, and afterward much decreased, with a corresponding increase in the liver, the muscles, and the brain.[57] Selmi found volatile phosphorous compounds in both the urine and the brain, and all were toxic.[48] Hemoglobinuria is described by Silbermann.[51]

Post-mortem the lesions are evident in a certain relation to the amount and form of the poison ingested and the duration of life afterward. The skin and conjunctivæ may be jaundiced and petechial hemorrhages in the integument are common. The muscle of the heart may be pale, or yellow and friable,[31] and the cardiac lesion may be seen as early as the third day.[19] The intima of the vessels may be in fatty degeneration, and rhexis of small vessels is common and may be general. Klebs found the vessels of the subcutis in fatty degeneration, and Orth and Heschl have described the same lesion in the brain. Hemorrhages, usually of small extent, may occur in any organ and in the muscles, including the tongue, heart, and uterus, and also under serous and mucous membranes. Extreme congestion of the liver, kidneys, ovaries, spleen, uterus, and other organs has been reported. Peripheral gangrene has been noted by Ehrlich, Heberda, Vollbracht, and others. The blood after death may be incompletely coagulated, which is explained by Corni and Ansiaux as due to lack of fibrinogen, fibrin ferment, and prothrombin. Heberda contradicts this view, though it is supported by experimental studies of Fränkel and Rohmann.

The lungs may not be altered apparently, but small hemorrhages under the pleura and throughout the organ are

not uncommon. Wegner found fatty degeneration in the tissue of the lung in chronic cases. Puppe, Carrara, and others describe fatty embolism of the pulmonary capillaries. (Cited in 61.)

The spleen is variously described as firm and congested, enlarged and soft, or practically normal. The Malpighian bodies may be prominent on the fresh section. Hessler claims that seventy-five per cent of all cases have enlargement of the spleen.

The liver invariably shows lesions after toxic doses of phosphorus. Since Rokitansky in 1859 and Hauff in 1861 first called attention to the hepatic effects of phosphorus, all observers have agreed in describing fatty lesions of the organ in such cases. Some of the more enthusiastic have described in addition early and rapid proliferation of periportal connective tissue, a lesion developed experimentally over long periods by Wegner, but which in the rapidly fatal cases in the human subject may be as truly a coincidence as the arterial hypoplasia described by Manner in cases associated with gangrene of the extremities. In gross the liver may be large, with swollen and blunt edges. In consistence it may be firm or impressible, mottled red and yellow, congested, anemic (Burgl), or with only the average amount of blood (Heberda). Frequently it is stained with bile. In older cases there may be diminution in the size of the organ, indistinctness of the acini, and red areas where destruction of the cells has left the turgid vessels surrounded by detritus. This form resembles the liver of acute yellow atrophy. An excess of fat may be present early, distributed all through the acini or confined to the peripheries, where degeneration is apt to begin. Microscopically the amount of fat varies, but in the highest grade there is complete destruction of the cells. The bile capillaries may be found empty and pervious (v. Pastau), or plugged with mucus (v. Ebstein and Bollinger), and the mucus plug may have become hyaline. Kohts, and Munk and Leyden agree in ascribing the jaundice to extension of inflammation from the duodenum and plugging of the biliary capillaries. Meyer[35] found, beside occlusion of

the finer biliary passages, plugging of the common bile duct also, and diffuse periportal proliferation of connective tissue.

The kidneys may be large. Cayley[8] in one case gives their weight as eleven and three-quarters ounces together. They may be congested or pale or yellow. The cortices are usually swollen and opaque, or distinctly yellow. Burgl[7] gives them as swollen but not opaque, with prominent glomeruli, in one of his cases. Jaundice and ecchymoses are often found in this organ. Microscopically, in early cases the epithelia of the convoluted tubules are fatty; later the cells of Henle's loops, of the straight tubes, and even of the capsules of the glomeruli may be fatty (Meyer, Virchow's Arch, xxxiii). The tubules may be more or less completely filled with desquamated fatty epithelia, leucocytes, which also may contain fat, bile pigment, and hyaline casts.

The mucous membrane of the stomach, apart from small hemorrhages, may be uniformly pale and swollen, cloudy and opaque, and white, gray, or yellow. Superficial erosions are described by some observers, but it is possible that post-mortem softening of the superficial layers of the mucosa may have been confounded with ante-mortem conditions. Microscopically the cells of the glandules are so swollen that their lumina are obliterated. These cells are opaque and granular, of a light yellow gray color by transmitted, and white by reflected, light. Acetic acid does not clear them. This lesion constitutes the gastradenitis phosphorica of Virchow[60] and is found in about one-half of the cases. It may be confined to either the pylorus or the cardiac end of the stomach, or be general.

The intestine may share in the inflammation or escape entirely. Superficial and punctate hemorrhages have been seen, Peyer's patches and the solitary follicles may be swollen, and the glands of Brunner and of Lieberkühn may resemble those of the stomach. The mesenteric nodes may be hyperemic and swollen (Coupland[11]).

The pancreas is usually in a condition of fatty degeneration.

One of the most recent studies of phosphorus, in relation

to its effect upon metabolism in man, was published by Pecci.[11] He finds that in severe cases of acute phosphorus poisoning the nitrogen excreted as urea is decreased; that the nitrogen excreted as ammonia is increased, in direct proportion to the amount of proteid destruction in the body; and that the nitrogen lost as uric acid is increased both absolutely and relatively. The last of these observations is explained as due in part to destruction of tissue rich in nuclein. The phosphates in both urine and feces are increased, the chlorides vary with the amount ingested in the diet. The sulfates vary with the degree of proteid destruction, keeping pace with the nitrogen. The conclusion is reached that phosphorus causes a great increase in catabolism, and that there is an active formation of ammonia in the body to counteract this.

The acute lesions obtained by experiments upon animals do not differ essentially from those noted for man. Besides the various fatty changes and the ecchymoses already mentioned, Puppe found the fatty contents of the blood increased in a dog fed with phosphorus, and "free fatty crystals" may be seen. The same is noted by Menard, Wegner, and Corni and Ansiaux. Krönig after feeding phosphorus to a dog for thirty-six days found fatty degeneration of the liver, with dilatation of its vessels and bile ducts, and after seventy-nine days, hyaline and fatty degeneration of the hepatic vessels. After one hundred and six days the liver cells were vacuolated, their nuclei broken down or absent, foci of leucocytes and detritus of hepatic cells appeared, and increase of the periportal tissue. Lebedeff asserts that the fat in the liver is brought to the organ, and is hence a fatty infiltration, but his experiments are without value. Leo thinks that although the liver gains, and other parts of the body lose in fat, the total in the body is increased; consequently there must be some production. Leisering studied four fowls, two males and two females, accidentally poisoned by phosphorus, finding little beyond congestion in the organs of the former and marked fatty changes in the latter. Since the males were spare and the females were fat he argues that the fat was carried to the

liver. But he reports that the kidneys in the females were fatty, and does not claim such a transport for these organs. Senftleben gave about 0.03 gram of phosphorus to a rabbit by injection, and on the following day about 0.015 gram by the mouth. Death occurred on the next day and already the myocardium was fatty and without striation, and there was beginning fatty degeneration in the villi of the intestine and in the kidneys. Wegner by careful feeding experiments with dogs, cats, and rabbits, carried on for several months, found the gastric mucosa swollen to as much as three times its normal thickness, indurated, and of a brown color, especially in the fundus, with elongation of the glands and increase of the stroma. The liver, beside the customary fatty changes, showed interstitial hepatitis "in optima forma." Silbermann injected phosphorus oil subcutaneously in dogs and obtained multiple autochthonous thrombi in various organs and peripherally. His opinion was that such thrombi might have a causal relation to the parenchymatous degenerations so characteristic of phosphorus poisoning. He also found marked hemolytic effects, the red cells becoming swollen and taking on various distorted forms, and the white cells becoming less numerous and more or less fragmented. Somewhat similar effects were observed by Fränkel and Rohmann in experiments on fowls. Taussig describes "an enormous destruction of the red cells" in fowls. Briliant found that both by injection and feeding experiments the blood pressure was reduced by phosphorus, but even more by PH_5. Falck states that subcutaneous injections of phosphorus in oil cause great increase in general metabolism in animals, with increase in the activity of the kidneys and the amount of the urine, decrease of the activity of the lungs and skin, and that from 54.4 to 97.6 per cent of the phosphorus injected was eliminated by the urine as P_2O_5. Taylor (Journ. Med. Research, Vol. IX., No. I., p. 59) finds that an essential action of phosphorus is its liberation of fat from combination with proteids, reversing "a normal metabolic relation." The result of such a proteolysis is the appearance of free fat in the cell.

EXPERIMENTAL.

In our experiments, dogs I. and II. were given the following doses of P_4S_3:

	Dog I.			Dog II.		
Day.	A.M.	P.M.	Daily total.	A.M.	P.M.	Daily total.
	Gram.	*Gram.*	*Gram.*	*Gram.*	*Gram.*	*Gram.*
1	0.218	0.000	0.218	0.217	0.000	0.217
2	0.046	0.040	0.086	0.042	0.043	0.085
3	0.051	0.063	0.114	0.053	0.045	0.098
4	0.045	0.064	0.109	0.048	0.057	0.105
5	0.052	0.065	0.117	0.048	0.075	0.123
6	0.030	0.023	0.053	0.053	0.067	0.120
7	0.097	0.135	0.232	0.136	0.154	0.290
8	0.142	0.135	0.277	0.149	0.112	0.261
Totals	0.681	0.525	1.206	0.746	0.553	1.299
Total, first period.....			1.206	.....		1.299

After the lapse of a week a second period of dosage began, as follows:

Day.	Dog I. A.M.	Dog I. P.M.	Dog I. Daily total.	Dog II. A.M.	Dog II. P.M.	Dog II. Daily total.
	Gram.	*Gram.*	*Gram.*	*Gram.*	*Gram.*	*Gram.*
1	0.128	0.085	0.213	0.106	0.090	0.196
2	0.092	0.093	0.185	0.084	0.098	0.182
3	0.073	0.078	0.151	0.086	0.064	0.150
4	0.076	0.073	0.149	0.134	0.082	0.216
5	0.055	0.067	0.122	0.060	0.059	0.119
6	0.096	0.099	0.195	0.096	0.107	0.203
7	0.118	0.092	0.210	0.068	0.100	0.168
8	0.096	0.097	0.193	0.101	0.109	0.210
Totals	0.734	0.684	1.418	0.735	0.709	1.444
Total for second period			1.418			1.444
Brought forward			1.206			1.299
Total amount received			2.624			2.743

Average daily dose, first period,	0.15075	0.1623
" " " second period,	0.1772	0.1805
" morning dose,	0.0884	0.0925
" evening "	0.0753	0.0788

Dog III. received single large daily doses for four days, as follows:

First day	1.000 gram.
Second day	1.516
Third day	1.600
Fourth day	1.845
Total	5.961
Average daily dose	1.4902

Dog IV. received a single dose of 10.0 grams.

Each dog received the following daily food in two portions:

Meat	100 grams.
Crackers	40
Lard	30
Water	430 c.c.

At the beginning of the first period the weight of dog I. was 7,820 grams, and at the end 7,707, being a loss of one hundred and thirteen grams. Dog II. at first weighed 6.880 kilo and at the end 7.480, showing a gain of six hundred grams. The total daily amount of the urine and its specific gravity could not be determined, as the animals could not be catheterized. The cages were washed down with distilled water and the urine made up to a definite amount (500 c.c.), and aliquot quantities of this were taken for analysis.

The feces were dried to constant weight. Before medication the daily amount of feces was about eight grams in the moist condition. After administering the tetra-phosphorus tri-sulfid the daily amount of feces increased, averaging fifteen grams for the first period. In consistence the feces were fluid, and at times there was slight diarrhea. Dog I. lost a little blood from the bowel on one occasion; this was due to his having swallowed a bit of wire in his food. When the P_4S_3 was stopped, the daily amount decreased, but rose again during the second period. Dog II. suffered from diarrhea on stopping the drug. The attack lasted but a day and the amount of feces was fifty-five grams. The average amount was higher throughout the second period and the average daily dose was also higher. The curve of the daily totals of feces thus shows a steady upward movement.

METABOLISM OF NITROGEN. — The total nitrogen in the diet was 5.88 grams. In the case of dog I., for the two weeks preceding medication the amount of assimilated nitrogen approached the available total without actually reaching it except on the eighth; and the daily variations were all above the level of five grams except once, on the eleventh. It is therefore safe to say that of the available 5.88 grams

of nitrogen only 0.88 gram were not assimilated. This continued for three days after beginning the administration of phosphorus tri-sulfid, until, in other words, the animal had received 0.418 gm. of the drug. From that point there was a distinct lessening of the nitrogen assimilated, a recovery at the end of the first period, and a second fall which lasted through the same period.

The corresponding curve for dog II. represents a similar fall during and after the first period, a recovery during the pause, and a lower average afterward. The fluctuations were marked after the end of the second period, reaching the lowest level of the whole series on the fifth day after discontinuing the doses.

The curve of nitrogen balance was kept for dogs I. and II. and showed practically a balance before medication, although the nitrogen was not totally assimilated. Then, with the beginning influence of the phosphorus compound, the slight apparent deficiency was exaggerated, and after stopping the drug the dogs had not nearly returned to a condition of nitrogen balance. In further experiments the animals will be kept to see when they return, if ever, to an equilibrium.

In the case of dog II. the amount of nitrogen in the feces was charted. At the end of each administration period there is a decided increase in the amount recovered. The curve persists on a high level for ten days after stopping the drug.

These variations in the assimilation of nitrogen may be explained in part by the lesion in the alimentary canal, which undoubtedly impaired the absorbing power of the mucous membrane, but the synchronous destruction of proteid in the formed elements of the body must also be taken into account.

The metabolism of phosphorus during the first period for dog I. rose steadily, as follows:

Dog I., Day	1	0.05 gram.
	2	0.10
	3	0.18

Dog I., Day 4	0.15 gram.
5	0.23
6	0.22
7	0.20
8	0.28
Second day of pause	0.38

From the latter point it fell through the remainder of the pause and the first days of the second period. In the case of dog II. the trend of the curve was upward, from 0.20 gram at the first to 0.44 gram on the second day of the pause. The observations for both dogs were untrustworthy for the second period, owing to inaccurate collection of the material, and are not given.

METABOLISM OF SULFUR. — For both dog I. and dog II. the fluctuations in the total sulfur contained in the urine during twenty-four hours were marked before the administration of P_4S_3, with a general upward trend for the ten days preceding medication. The highest point reached, from a start at 0.16 gram for both dogs, on the eighth, was 0.261 five days later for dog I., and 0.212 nine days later for dog II. In each period of treatment there was an evident fall, but in the first period this occurred during the beginning with each dog and in the second toward the end of the period. Between, there was a recovery, which was well marked at the end of the first period, lasting through the pause and the early half of the second period.

The general course of the curve for ethereal sulfur is upward, beginning with the first medication and rising steadily through both periods and the pause between. Dog I. shows the following figures, given in tenths of a gram, on alternate days through the whole twenty-two days:

Dog I., Day 1	2.3
2	1.0
4	4.0
5	1.8
7	3.4
9	2.3

Dog I., Day	11	3.4
	13	3.0
	16	4.8
	18	8.3
	21	10.1

A similar but less pronounced rise was evident in the curve of ethereal sulfur in the case of dog II. With this dog there was a fall after stopping the second period, the curve running down for the next ten days. On the whole, the variations in the excretion of ethereal sulphates were of more restricted range than those of alkaline sulphates, both actually and relatively.

METABOLISM OF FAT. — The disturbance in the metabolism of fat was even more marked than in the case of nitrogen. The feces often contained masses of undigested fat on gross examination. The curve of fat assimilated underwent great fluctuations, and the curve of unassimilated fat, excreted as such in the feces, rose steadily for dog I., and during the second period for dog II. On the whole, these characters were less evident for dog II. than for dog I. The daily record of fat in the feces of dog I. is as follows:

Dog I., Day	1	0.8 gram.
	2	3.4
	3	1.0
	4	0.8
	5	8.0
	6	8.0
	7	4.0
	8	8.4
		Pause of one week.
	9	3.0
	10	3.2
	11	1.2
	12	1.2
	13	3.6
	14	1.0

	Second Period.
Dog I., Day 16	11.0
17	12.0
18	4.4
19	4.4
20	8.4

For dog II. the variations ran from 2.0 grams on the first day to 7.4 grams on the fifth day of the first period. After stopping the drug the tendency was to return, in each case, to the original low level, without ever quite reaching it.

It is interesting and important that neither albumin, sugar, leucin, nor tyrosin was ever detected in the urine of any of the dogs.

Sevéne and Blanc report that animals exposed to the vapor of tetra-phosphorus tri-sulfid succumb sooner or later. The fifth experiment consisted in covering a rabbit with a large bell jar, through which air was passed at the rate of five hundred cubic centimeters per minute. All the air thus passed through went first through five layers of the compound, alternating with layers of absorbent cotton, and then through two water bottles containing respectively solutions of acetate of lead and nitrate of silver. Before passing through the compound the air went through a wash bottle containing water, and was saturated with moisture. After several hours in this apparatus and under these conditions the rabbit showed absolutely no effect. The bottle containing the nitrate of silver was then removed, so that the respired air contained any volatile compounds of phosphorus which might be taken up. Nothing was noted beyond slight acceleration of the respiration. The animal was quiet and fed readily. The bottle containing acetate of lead was then removed, so that the animal breathed any volatile compounds of phosphorus or sulfur which might have been absorbed. The condition of the animal remained normal, and after these observations had been carried on over three days they were abandoned.

PATHOLOGICAL.

The dogs were killed by puncture of the medulla, except the fourth animal, and the organs were put in hardening fluids immediately. The records of the gross and microscopical examinations were as follows:

Necropsy of male dog No. 1, Dec. 10, 1901, weight 7 7 kilo.

Animal well nourished. General inspection of the abdominal contents discovers nothing abnormal.

Liver. Color pale, consistency normal, markings of acini distinct.

Kidneys. Capsules peel easily, surfaces smooth and pale, cortices pale, slightly swollen, bulge a little above the section, markings indistinct, glomeruli visible as minute red points.

Spleen. Normal in color, size, and consistence.

Pancreas. Pale and anemic.

Mesenteric nodes distinctly swollen and pink.

Stomach. Slight congestion in fundus, contains a small amount of undigested food, mucosa apparently normal.

Duodenum. Mucosa thickened and bile stained, surface of small intestine throughout covered with thick, frothy mucous; agminate and solitary follicles prominent, being slightly edematous: no fecal odor in any part of intestine.

Heart in valves and myocardium apparently normal.

The liver on chemical analysis contained 6.25 per cent of fat.

Microscopic Examination. — Liver. The cells in the peripheries of the acini are in a state of fatty degeneration, and yet, on the whole, even here the nuclei are well preserved and take the stain freely. In places the protoplasm is reduced to a mass of detritus and free nuclei. The bile ducts are normal. There is no excess of connective tissue.

Pancreas. The cells are everywhere swollen and the nuclei are vesicular; the protoplasm is slightly acidophile. The islands of Langerhans appear normal. The cells of the ducts are swollen, and this decreases their lumina appreciably.

Kidney. There is some active hyperemia of the cortex, but not of severe degree. The epithelia of the cortical tubes for the most part are well preserved, only here and there the nuclei are vesicular, and there is cell detritus in the lumina of the tubes. The Malpighian bodies and the looped tubes are normal.

Spleen. The organ is generally hyperemic and a few small hemorrhages are found. The lymphadenoid bodies are slightly hyperplastic.

Lung. In general the organ is hyperemic and edematous, but not to a marked degree. In some areas there is hemorrhage into the alveoli, and about these are small thrombi of red cells in the capillaries.

Small intestine. In the villi there is a slight active hyperemia, the investing epithelia are well preserved, and there is an apparent excess of beaker cells.

Mesenteric node. Both hyperemic and edematous.

Heart. The vessels of the myocardium are congested, the muscle fibers are opaque, and their striation is indistinct, but the nuclei are generally well defined and take the stain well.

Prostate. Normal.

Necropsy of male dog No. II., Dec. 21, 1901.

Dog well nourished; weight 7.48 kilo.; the omentum contains a large amount of fat.

Liver. Very much congested, but apparently normal in structure.

Kidneys. Large, capsule peels readily, the surface is smooth and of a mottled red color. On section the cortex is swollen and the markings are indistinct.

Spleen. The lymph-adenoid structures are very prominent, otherwise the organ is normal.

Pancreas. The organ is small and congested and there is an apparent increase in its stroma.

Stomach. Full of undigested food and the mucosa shows slight general (functional) hyperemia.

Small intestine. The mucosa is thickened and covered with an excess of mucus. In the lower portion there is some pigmentation with bile and the same excess of mucus. No fecal odor observed throughout the intestine.

Heart. Normal.

Microscopic Examination. — Liver. The protoplasm of the hepatic cells is coarsely granular or actually lacking in many places, but the nuclei persist and take the stain well. This is true of the entire acinus. The capillaries in the acini are hyperemic. There is no pigmentation of the organ. The biliary capillaries are pervious and their epithelia are normal.

Kidneys. There is acute arterial hyperemia throughout the cortex, and in its outer zone there is much granular degeneration of the epithelia. In the lower zone, just above the pyramids, and in the columns of Bertini, the tubes present either a granular epithelium whose nuclei are well preserved, or the cells are beginning to break down.

Spleen. The lymphoid tissue is copious and proliferating; elsewhere there is much granular brownish-yellow pigment, both free and enclosed in leucocytes.

Pancreas. The protoplasm of the cells is distinctly acidophile and finely granular; the nuclei are distinct and stain well, but are generally vesicular and have their chromatin disposed in minute granules; the nucleoli are large and prominent. The vessels are normal, there is no increase of connective tissue, and the epithelia of the ducts are slightly swollen but not granular.

Small intestine. The investing epithelium shows an excess of beaker cells. The core of each villus presents a dilated lymph channel; and the

solitary follicles are large and their lymphatics are wide and contain dark amorphous pigment, both free and in leucocytes. The leucocytes of the follicles have scanty granular chromatin in their nuclei. Some villi are slightly hyperemic.

Heart. Striation of the muscular fibres well preserved, no fatty degeneration or hyperemia noted.

Adrenal. The protoplasm of the cells in the middle and outer zone is marked by fine vacuoles; in the central zone there is little vacuolation but some hyperemia.

No trace of hemolysis is observed in any of the vessels of the organs examined.

Necropsy of male dog, No. III, Dec. 5, 1901. Well nourished, weight 6.6 kilo.

Liver. The organ is large and congested, and the section is mottled by areas of fatty infiltration. Elsewhere the peripheries of the acini are light colored and clearly defined, marked here and there by minute yellow collections of fat. The gall bladder contains orange-yellow bile with small black grains of pigment.

Kidneys. Capsule strips easily, surface generally smooth, cortex swollen, and markings indistinct.

Spleen. Congested and marked by prominent lymphadenoid structures.

Pancreas. Large and pale.

Mesenteric nodes swollen and prominent.

Stomach. Contains undigested food and is slightly congested.

Small intestine. The mucosa throughout is swollen and covered by thick, frothy mucus, which is mingled with bile. There is no fecal odor. The solitary and agminate follicles are edematous.

Heart. Normal

Prostate irregularly swollen and varies in color on section from light yellow to pink.

Microscopic Examination — Liver. The central zones of the acini are hyperemic, the red cells being of perfect contour. The epithelia show marked granular degeneration, in places represented only by detritus, where remaining nuclei are large and have prominent nucleoli. There is but little pigmentation. The epithelia of the gall ducts are slightly swollen and their nuclei are large and vesicular.

Kidneys. There is active hyperemia of the cortex. The epithelia of the convoluted portions are swollen and in places block the lumina, in other places the granular protoplasm has broken down and left slender protoplasmic strands and bridges across the tube. In some tubes the epithelium is wholly desquamated from the wall. The glomeruli as a whole are shrunken, and in places there is a proliferation of the cells of the capsule of Bowman. The great collecting tubes contain cells, fatty epithelia, white cells, and granular detritus. The epithelia of Henle's

loops are elongated and distorted. The coats of the arteries are thickened, especially the media.

Spleen. The cells of the Malpighian corpuscles are apparently hyperplastic and their nuclei are very large; otherwise the organ is hyperemic, and contains an excess of granular pigment, mostly extracellular.

Pancreas. There is marked eosinophilia of the central portions of the acini; the nuclei are large, and the nucleoli are prominent. The protoplasm of the cells is granular and in places vacuolated. The distinction between cell and cell is not always clear; they seem to be partly fused in places. The epithelia of the finer ducts are somewhat swollen.

Mesenteric node. Edematous, and the lining cells of the main lymphatic channels are swollen and project into the lumen.

Small intestine. The villi show many beaker cells among the investing cells, the core of the villus is edematous, and the lymph channel is dilated.

Stomach. The chief cells of the glandules are slightly swollen and granular. The superficial epithelia are well preserved and have a clear protoplasm.

Heart. Striation is partly lost, the fibers are a little swollen and their nuclei are irregular and distorted.

The liver on chemical analysis contained 4.14 per cent fat.

Necropsy of male dog, No IV., weight about 6.5 kilo. General nourishment good. Animal killed by section of great vessels of neck. General appearance of organs in consequence bloodless.

Liver. Pale, central vessels dilated, slight bile staining in places.

Kidneys. Normal in size, capsule strips imperfectly, surface pale and presents a few cysts. In section the cortex is slightly swollen and the glomeruli are visible.

Spleen. Slight hyperplasia of lymphadenoid elements.

Pancreas. Pale, but apparently normal.

Mesenteric nodes. A few of them are enlarged and edematous.

Stomach. Physiological hyperemia; contains undigested food.

Small intestine. Lymphoid structures throughout edematous and prominent. In the lower third the mucosa is congested and bile stained, and the colon presents a few very small submucous hemorrhages.

Microscopic Examination. — Liver and bile ducts normal, not more fat in the peripheries of the acini than normal, slight biliary pigmentation.

Kidneys. Cloudy swelling.

Spleen. Slight lymphoid hyperplasia and excess of amorphous pigment; otherwise normal.

Pancreas. Protoplasm generally intensely acidophile and markedly granular. Islands of Langerhans hyperemic. Epithelia of ducts swollen to partial occlusion of the lumen in places.

Mesenteric node hyperemic and edematous.

Stomach. Normal; lymphatics very wide.

Small intestine. Villi profusely infiltrated by mononuclear leucocytes.

Adrenal. Central zone hyperemic. Protoplasm generally finely vacuolated.

Testis. Normal.

It is evident from these gross and microscopic lesions that the action of tetra-phosphorus tri-sulfid is wholly unlike that of phosphorus, though the assertion of Courtois-Suffit, that it is "not injurious, no matter how large the dose," cannot be accepted as accurate. Its effect is that of a mild irritant upon the structures with which it comes in contact, like the intestinal villi, but its action is more severe after absorption through the intestinal wall. The reaction in the mesenteric nodes is a mild exudative inflammation. It may reach the heart through the lymph stream or by way of the portal and the hepatic veins, but when circulating through the vessels of this organ it may cause only a local hyperemia and a cloudy swelling. This may be contrasted with Senftleben's experiment with phosphorus, in which a rabbit received about 0.045 gram in forty-eight hours, died the next day, and showed already marked fatty degeneration of the heart.

Carried to the liver in large enough doses, or over a long enough period of dosage, the compound may cause the epithelia to break down. This occurs first at the periphery of the acinus, but may involve its whole extent; in that case the lesion is less marked about the central vein. Even when the protoplasm of the cells is much affected the nuclei persist and take the stain well. Probably but little is excreted by the liver, or else its nature is so modified that it causes no appreciable lesion of the biliary epithelium. The cells in the bile ducts were not so much affected in any case as the epithelia of the pancreatic ducts, and in the latter the change consisted only in swelling and slight granular alteration.

The organ which probably excretes the major portion of the drug is the kidney, and the severity of the effect is here most pronounced, but one must be struck with the fact, even

here, that the enormous doses, compared with phosphorus, produce surprisingly little effect, and also that the nuclei persist for a time after the protoplasm of the cell has been lost.

The form in which the tetra-phosphorus tri-sulfid circulates in the blood is undetermined, but it is notable that it causes no hemolysis.

The relation between the lesions and the symptoms, including the records of metabolism, is sufficiently clear and needs but little explanation.

The increase in the daily amount of feces during administration, the decrease in the assimilation of nitrogen (dogs I. and II.), the increased N in the feces (dog II.), and the increase in the fat recovered from the feces, can all be explained by the hyperemia and increased production of mucus along the alimentary tract. The lesion was hardly severe enough to be considered more than a mild catarrhal inflammation. The steady rise in the assimilation of phosphorus shows that absorption from the intestine was still going on, and the fall in the nitrogen balance, together with the lesions in distant organs, as noted, confirm this idea. Some of the increase in excreted nitrogen is due to the protoplasmic destruction, but the proportion has not yet been determined.

It is an important fact that no one of the dogs died, though receiving doses which, if phosphorus, would certainly have been fatal in all four cases. As soon as the drug is stopped there is an immediate and buoyant recovery. Of the four dogs, possibly No. I. would not have recovered ultimately, the others probably would have done so. Assuming that the other three would have recovered, and calculating proportional doses for an adult human being, we have the following statement of the relative toxicity of the sulfur compound as compared with pure phosphorus:

Dog II. received	2.624 grams	in 22 days	} equivalent doses for a man weighing 60 kilo.	{	22.88 gram.
Dog III. "	5.061 "	" 4 "			63.28 "
Dog IV. "	10. "	" 24 hours			90.9 "

Taking Kolisko's higher figure as the lethal dose of phosphorus, namely, 0.20 gram, it is evident that P_4S_3 is one hundred times less toxic than phosphorus on the least favorable showing, and over four hundred times less toxic according to the last experiment. In other words, no amount of tetra-phosphorus tri-sulfid, which any human being could take by accident or design, would probably prove fatal.

CONCLUSION.

1. Inhalation of air, passed through tetra-phosphorus trisulfid in substance, has no appreciable effect upon the rabbit at room temperature.

2. In the dog P_4S_3 acts as a mild local irritant upon the alimentary canal.

3. This action is more pronounced in the small intestine than in the stomach.

4. In the circulation it acts destructively upon the protoplasm of parenchymatous cells.

5. The effect varies from a strongly acidophile character in the protoplasm to its conversion into detritus.

6. The nuclei of the parenchymatous cells show a marked resistance, remaining and taking the stain even after the body of the cell has disappeared.

7. The effect is most marked in the epithelia of the renal cortex, of the liver, and of the pancreas, and in the muscular fibres of the heart, in the order named.

8. The destruction is manifested after long treatment with small doses or shorter periods of heavy dosage.

9. Perhaps the rapid recovery of the animals after stopping the administration is connected with the persistence of the nuclei.

10. In general the agent increases catabolism by this action upon epithelia, while at the same time lessening anabolism by its local and alimentary effects.

11. It does not cause hemolysis, nor jaundice, nor the appearance in the urine of albumin, sugar, tyrosin, nor leucin.

12. Consequently tetra-phosphorus tri-sulfid may be considered non-toxic in any ordinary circumstances.

(NOTE. — Our thanks are due to Mr. C. D. Partridge, B.Sc., for valuable assistance in the preliminary work connected with this investigation.)

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"WHITE-SPOT DISEASE."

By James C. Johnston, M.D., New York, and Samuel Sherwell, M.D., Brooklyn.

(from the pathological laboratory of cornell university.)

THE patient is Dr. Sherwell's. She was presented for a diagnosis of her disease to the New York Dermatological Society, and I suggested that she submit to a pathological examination. The photographs are the work of Dr. B. H. Buxton and his assistant in the photographic department of Cornell, to whom our best thanks are due.

History: The patient is a woman twenty-six years of age, born in Ireland. There is nothing worthy of note in the family history. She herself enjoys fairly good health, but is thin and evidently neurotic, with a poor physical development; "a human weed," in Dr. Sherwell's apt phrase. She has suffered from time to time with evanescent erythematous eruptions affecting the face chiefly, which have, however, nothing to do with the present condition.

The lesions which form the subject of this paper began thirteen years ago; her first menstruation did not appear until some months later. According to the patient's statement, the number of lesions has gradually increased, while they have changed very little individually. The area most characteristically affected is the anterior surface of the chest, extending from the outer third of the left clavicular region to the middle third of the right, and from the shoulder girdle to the upper margin of the breasts. There are in addition striate atrophic spots about the root of the neck and over both scapulæ; perhaps the terminal stage of older lesions. The patient's statement is not clear on this point.

The spots on the chest are arranged like beads in the successive rows of a necklace, following the skin lines and roughly corresponding to the distribution of the vascular supply, running across from one side to the other. The individual lesion is raised only very slightly above the surface, and is smooth to the touch. The smallest, presumably the earliest, are no larger than the head of a white pin. The outline is sharp and irregular, without a trace of inflammatory areola, running often into tiny projecting points as it increases in size. There is not much tendency in the lesions to unite, but when this occurs, the

spots form an end-to-end juncture, so that they are never of great extent. Certain of them exhibit a central point which clinically gives the appearance of umbilication, but in reality is a tiny elevation due to a separation of the epidermis (see histology). The color is by far the most striking feature of the disease. It is the dead-white of snow. The chest looks as though it had been spattered with a white-wash brush.

In the course of time, to which no definite limit can be set, certain of the spots undergo involution. A thin scale separates and there is left an atrophic area corresponding exactly to the white spot which preceded it. This atrophy may be punctate or striate, and is not different, except in distribution and outline, from that resulting from lupus erythematosus, symmetrical atrophy, or ordinary *striæ atrophicæ*. There are no subjective symptoms, but the patient scrapes off the scales from time to time and rubs the other lesions from the annoyance their presence gives her.

Histology: A single patch was removed with a cutaneous punch from the left side just above the breast, including the entire diseased area so far as could be seen clinically. The lesion showed a central point which I took to be an umbilication, but which resolved itself into the vesicle-like formation shown in Fig. 1. Sectioning was done in paraffin and staining with hematoxylin-eosin, Van Gieson's picrofuchsin, Nocht, hematoxylin-orange-acid orcein.

The lesion is as nearly a pure degeneration as it is possible to imagine in the skin where processes are more than apt to show great complexity. It is limited to the papillary body and the upper portion of the reticular layer. There is no uniformity of distribution; the degenerated area stopping abruptly at certain points, to begin again, apparently in lesser degree, a little farther on. It reaches greatest development naturally in the central portion of the section which corresponds with that of the excised skin. The irregularity of distribution is in conformity with the clinical appearance of the spots noted above.

In the degenerated mass, the collagen has altogether disappeared, its place being taken by a granular material which has lost its characteristic acidophile staining tendency. It has little or no affinity for eosin, orange or fuchsin. There is no deposit of any foreign material like calcium salts, such as I expected to find. The elastic tissue is broken up into short lengths of fiber or granules which take the acid-orcein stain more deeply than the normal tissue. The elastic network covering the papillæ has completely disappeared where the process is

most advanced, and in other places shows beginning rupture. Some of the vessels forming the plexus at the base of the papillary body are preserved. They are loosened from their attachments to the surrounding tissue and are often dilated, doubtless a result of diminished tissue tension. The endothelial cells are swollen and project into the lumen. In places the lumen is perceptibly narrowed in this way; in others, there is complete collapse of the walls. These appearances, except endothelial swelling, may be artefacts. Thin paraffin sections are prone to them, but they are constant here even when there is no distortion in the section.

An irregular lymphocytic infiltration sheaths the vessels in places and in others seems independent of them. The number of mononuclear cells is very small. Proliferated and swollen fibroblasts are to be found here and there. These changes are shown in Fig. 2. The area of degeneration shades gradually below into the subcutaneous tissue.

The epithelial changes are secondary. Interpapillary projections disappear in places where degeneration is complete, and while there is nothing to prevent the growth of epithelial cells into the subjacent tissue, they do not proliferate. The rete is reduced to a few layers of cells, four or five, which show marked hydrophic degeneration. The horny layer is very slightly increased; the granular layer absent or reduced to the thickness of a single cell. The space between the epithelium and degenerated connective tissue, while it contains granular material which may be precipitated albumen and an occasional leucocyte, must be regarded, if not as an artefact, at least as accidental, possibly the result of rubbing of the clothes. (See Fig. 1.) Separation would naturally be very easy in such a condition. Fibroblast proliferation and lymphocytosis are evidently of no great importance in the process, so there remains only the degeneration of the connective tissue to account for the clinical appearance.

There is no involvement of the sweat apparatus, the only appendage present.

As to the cause of such a tissue change, one can only offer conjecture, but reasoning from analogy in other organs, so pure a degeneration ought to be due to local interference with nutrition through diminished blood and lymph supply. I believe that such is the case in this instance, and in spite of the fact that I cannot demonstrate it to my own complete satisfaction,—it is not a capillary thrombosis such as is found in erythematous lupus, but an obliteration of the smaller branches in the recticular plexus. The obliteration may be congenital or acquired, due to failure of complete canalization or

blocking by swollen endothelium. The latter seems more probable. This must occur only in the smallest vessels or the lesions would be larger. The irregularity of the spots is explained by their occurrence in the distribution of the small twigs.

There seems no reasonable explanation of the whiteness of the spots except one, a phenomena which is not unfamiliar. The degenerated skin held to the light is translucent, while in reflected light it appears perfectly opaque. I am at a total loss to understand why this should be so in white-spot disease and not in other atrophies. There may be some chemical difference in the products of the degenerative process; this, however, is beyond the question and hardly seems worth while to determine.

The disease should be classed with the atrophies, next to symmetrical atrophy, which, perhaps, closely approaches it. The name, "White-Spot Disease," which Westberg gave it in 1901, is striking and characteristic enough to deserve perpetuation.

Treatment: After the nature of the disease was determined, it was evident that the best result to be expected is an atrophic spot replacing the white appearance. It was obviously impossible to remove each spot surgically, and the only measure that remained to be considered was the use of revulsives strong enough to cause an active inflammation and so dissolve and carry off in the inflammatory serous and leucocytic exudate the remains of the degenerated fibers. I recommended the use of a saturated solution of resorcin in alcohol, applied three or four times a day. The result has been gratifying, and with a minimum of reaction; the white spots are on the verge of disappearance. In another case, I would use a more vigorous method, that which was recommended by McGuire, of Washington, in this Journal for the removal of xanthelasma of the lids, the application of crystals of trichloracetic acid, touching only the spots themselves. One application would doubtless suffice. The reason for the failure of Westberg's salicylic acid and bichlorid is that he used them in an ointment and not in sufficient strength.

Literature: The first case I ever saw was one exhibited in Chicago by Dr. F. H. Montgomery, in 1901, before the American Dermatological Association. I was not content even at that time with the generally expressed opinion that it was a peculiar form of morphœa. The correctness of that stand is obvious from my study of this case. The patient was a young woman, and the lesions corresponded in every particular with those I have described in both localization and appearance.

Westberg's case is the only one in the literature, although others must have been seen and called by other names. Even so, they have not been reported. The following is an abstract of Westberg's work (*Monatsch. für prakt. Dermat.*, 1901 Vol. XXXIII, p. 355). I am quoting from the *British Journal of Dermatology*, as I have not the reference at hand. The article has a photograph and a colored plate of the histology.

The patient was a girl of eleven years, strong and healthy. The spots appeared about two years previous to the observation, following measles. There were sparsely and discretely scattered about the thorax, hemp-seed to bean-sized, round and oval spots, chalky-white in color and set in normal skin. Their surface was smooth and they exhibited "playing-card" resistance to the touch. The spots on the whole corresponded to the lines of cleavage. There was a slight elevation when the skin was put on the stretch. There was no desquamation, no bullous formation, or other change in the spots. Histologically, Westberg found hypertrophy of collagen, an *increased* staining power of the affected fibers and numbers of mast-cells.

The clinical appearance of the three cases is plainly identical. The difference in histological findings is probably due to the fact that Westberg's case had lasted at most two years as against thirteen in mine. It is a common thing for the first change in a fibrous tissue degeneration to be a swelling of the fibers, followed after a variable period by a breaking up. In the early stage also they have an increased affinity for acid dyes. The chemical change must be identical in order to give the dead-white opaque appearance. I cannot understand his discovery of mast-cells in numbers. There was not one in my sections. The short duration of the disease also explains why he saw none of the exfoliation which sometimes occurred spontaneously in our case. It will undoubtedly occur after a time, and it is equally safe to predict that slight traumatism would separate epidermis from degenerated corium.

The introduction of a new disease hardly needs an apology when it is accompanied by a reasonable explanation of its nature and an efficacious method of treatment.

Fig. 1.

Fig. 1. Entire lesion (X 60). Separation of epidermis in vesicle-like formation. Degenerated, homogeneous-looking papillary body with sparse lymphocytosis below. Dilatation of lymph and blood spaces.

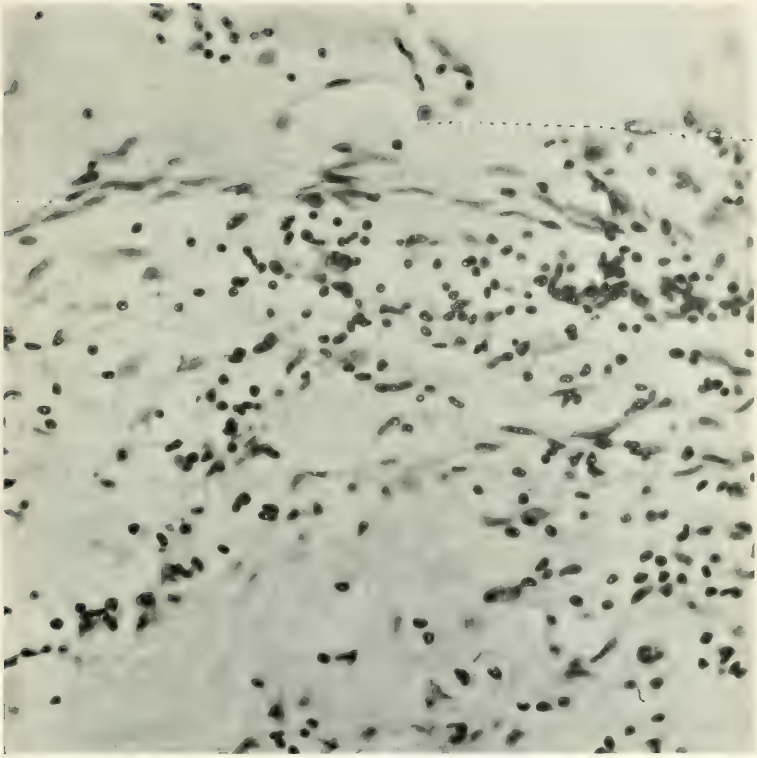

Fig. 2.

Fig. 2. X 200. Taken from border of space downward below place marked X in Fig. 1; *a*, obliterated capillary; *b*, dilated capillary lined with swollen endothelium. Degeneration extends very little below *a*. Lymphocytosis and fibroblast proliferation.

A CASE OF PAGET'S DISEASE OF THE NIPPLE.

By George Thomas Jackson, New York.

In September, 1896, I read before the American Dermatological Association the report of a case of malignant papillary dermatitis, together with the pathological report upon the specimen by Dr. J. S. Ely. [*Jour. Cutan. and Gen. Urin. Dis.*, 1896, xiv—428.] Dr. Ely regarded "the lesion in the skin as inflammatory in nature, due primarily and pre-eminently to a change in the corium of such a nature as to cause a very considerable connective tissue development, and, by interference with the nutrition of the skin, to lead to an intensely œdematous condition of the epidermis and of the papillary layer of the corium." He regarded the tumor formation in the gland as a "fibroma of the so-called pericanalicular variety," and that this was primary, while the skin lesion was secondary.

Since 1896 I have had several cases, but the one here reported is the only one from which I was able to obtain material for microscopical examination. As will be seen, the history of the case is meager. As the disease is a well-recognized one and the pathology is of more importance than the description, I venture to report it, together with the pathological study by Dr. Otto H. Schultze:

Mrs. J. L., æt 68. Widow.

The patient came to me on May 15, 1900. She stated that the disease had existed for nearly three years, and that the first symptom she noticed was a feeling of soreness under the right nipple and a few drops of blood. Since then the disease had been steadily progressive.

Examination showed that about the right nipple there was a red, raw patch about the size of a silver dollar; this was sharply defined, and gave to the touch the impression of infiltration into the tissues. The nipple had nearly disappeared, being drawn down into the breast so as to form a deep pit. The right breast was atrophied and all through it, but especially at the upper and outer aspects, there were hard masses. There were no appreciable enlargements of the axillary glands.

The patient's health was fair, though of late she had become weaker. There was no history of injury or anything to account for the disease. I advised her to have the whole breast amputated by a surgeon at once. On the 29th of May she was operated on by Dr. H. Packard, in a Boston hospital. The whole gland and a portion of the pectoral muscle were cut away, and the axillary glands were re-

moved, as they were found to be enlarged. Hard nodules were also removed from under the clavicle.

Dr. George E. May, her physician, reported that the patient made a good recovery from the operation. Three weeks afterwards she complained of nausea and distress in the abdomen. Her liver was found markedly enlarged. Ascites followed, with marked prostration. She became suddenly delirious and was dead in three days. She had no pain, icterus, or constipation. Vomiting did not occur until the last two days of her life.

Dr. Packard kindly sent me a portion of the diseased tissue, the results from the examination of which are given below:

PATHOLOGICAL REPORT UPON DR. JACKSON'S CASE OF PAGET'S DISEASE.

By Otto H. Schultze, M.D.,

Professor of Pathology in University of Vermont; Instructor in Pathology in Cornell University Medical College.

The portion of a breast, received from Dr. George T. Jackson, was about 6 cm. in length and 3 cm. in width and thickness. It was wrapped in gauze, soaked with 10 per cent. formalin solution, so that the skin surface was partly folded over the underlying tissue.

Beneath the subcutaneous adipose tissue, a firm mass presents on one surface of the specimen (evidently the surface of section from the rest of the breast), a fibrous connection with the skin. In this situation the skin is retracted, and presents a darker area adjoining the line of section and of round contour on the skin surface of the specimen. This area is covered with minute papillæ, and is traversed by several radiating fissures which nearly reached its margin.

A cross section through the middle of the specimen shows a marked thickening of the skin in the papillary area above described. The fibrous band of connection between the skin and the firm mass is quite narrow. The mass presents a bluish-white fibrous structure, with many firm, white streaks and dots, larger opaque, yellowish-white areas from 0.5 mm., in diameter, to 3 mm. x 1 mm., in extent, and a few cross sections of canals, the largest 0.5 mm. in diameter.

Microscopical sections were made by the celloidin method. The accompanying photographs of sections stained with hematein were made by Mr. John N. Lett.

The skin is normal up to the margin of the papillary area above described. From this point to the edge of the specimen, namely, the plane of section from the rest of the breast, the pathological changes

become more marked. (Fig. 1, from left to right.) These changes begin in both the epidermis and corium. In the epidermis the deeper prickle cells become enlarged and multiply. Many poorly fixed mitotic figures are found. The protoplasm of the cells diminishes in staining reaction, and clear spaces appear about the nucleus (Fig. 3). These spaces increase in extent so that finally the periphery of the cell alone remains. The nucleus is enlarged, vesicular, and pale, or presents a cloudy granular appearance. Where the degeneration of the protoplasm has advanced the nuclei are smaller, more densely stained, or broken up. Isolated cells are found retracted, and entirely or in part surrounded by a clear space. Some spaces of round contour are found, containing nuclear fragments or a few polynuclear leucocytes. The basal layer of the epidermis appears compressed, and its nuclei stain more densely. No evidence of active proliferation or invasion of the corium is found.

The changes described finally involve the entire layer of prickle cells. The granular and horny layers become irregularly thickened. The cells of the granular layer are larger and in places their granules are so dense that the structure of the cell is obscured, presenting thick, densely pigmented areas in the section (Fig. 3). The thickening of the horny layer is not so pronounced. The entire epidermis is thus thickened and the interpapillary projections become longer (Fig. 3).

With the advance of the degenerative process in the prickle cell layer, the horny layer disintegrates and becomes loosened. With its separation, the granular layer lies bare upon the surface and in turn is also lost. Finally the prickle cell layer becomes entirely disintegrated and disappears. The basal layer can be traced to the border of the ulcerated area, and upon its surface isolated fragments of this layer are found (Fig. 4).

A cross section of a fissure (Fig. 4) presents an appearance that might at first be mistaken for an invasion or down growth of the epidermis into the corium. On closer study it becomes quite clear that the fissure is due to a retraction of the skin. The epidermis presents throughout the thickness of the invaginated fold the changes already described. The basal layer is everywhere clearly marked off from the corium, and shows no evidence of invading it. The prickle cells above the basal layer are in a stage of degeneration, and so disintegrated in places as to leave the basal layer almost bare. The length of the fissures and their radiating arrangement is conclusive evidence of retraction.

In the corium the first change appears in the papillary layer (Fig. 3), and consists of an infiltration with lymphocytes about the vessels. Among the lymphocytes numerous plasma cells are found. This infiltration finally becomes so dense that the structure of the papillary layer is almost obscured (Fig. 4). As the process advances, an infiltration of the same character appears about dilated vessels in the deeper layer of the corium. Finally where the epidermis has completely disintegrated, a new growth of blood-vessels, surrounded by a dense infiltration of lymphocytes and plasma cells, appears in the corium (Fig. 5). In and near the area of ulceration polynuclear leucocytes appear in moderate amount. They also infiltrate the degenerating epidermis.

In the firm mass or tumor, the parts visible with the unaided eye and described as round yellowish, or opaque white dots and streaks and some as canals, are found to be the cross and longitudinal sections of lactiferous ducts. (Fig. 1.)

They are markedly increased in size. Their walls show an epithelial lining of four and more cells in depth. These cells are increased in size, are round or polygonal from mutual compression. The outermost are more markedly compressed and stain more deeply. They show no evidence in the larger ducts of breaking through the surrounding elastic tissue. The inner cells show degenerative changes in the appearance of a clear zone about their nucleus. The nucleus becomes smaller and stains more deeply.

In some ducts the lumen is quite large and empty, in others it is filled with epithelial cells showing all stages of degeneration to complete disintegration into a granular detritus.

In some of the larger ducts, small papillary projections of epithelial cells into the lumen are found.

In other ducts the lumen is narrowed by the thickened epithelial wall.

The stroma of the breast is densely fibrous, and in areas adjoining the ducts presents an infiltration of lymphocytes and plasma cells about the vessels.

In the smaller ducts (Fig. 2), the epithelial cells show a distinct appearance of invading the surrounding tissue.

In a few places groups of alveoli about the terminal ducts show an increase in size and proliferation of their epithelial cells, with loss of the lumen. The outermost cells are more densely stained and compressed. In the deepest part of the breast adjoining the muscle, unchanged ducts and alveoli were found.

The changes described in the skin, namely, infiltration in the papillary layer, proliferation and degeneration of the epidermis, beginning in the deeper layer of the prickle cells, and finally proceeding to complete ulceration, are characteristic of Paget's disease or malignant papillary dermatitis. The character of the process is distinctly inflammatory. The lesion differs as widely from eczema as it does from epithelioma. Its differentiation from acute eczema is so patent that it need not be discussed here. From chronic eczema, to which this disease has so frequently been likened, and with which it has so frequently been confused, it differs in the subsequent degenerative character of the process ending in complete ulceration.

Following the primary proliferation of cells in the deeper layer of the rete mucosum, and the thickening of the epidermis, the interpapillary projections of the epidermis increase in length, with a corresponding enlargement of the papillæ. An actual invasion of the corium does not occur. There is no evidence of active growth in the basal layer of the epidermis. On the contrary, with the subsequent degeneration of the deeper prickle cells, the basal layer appears compressed. The final complete ulceration of the epidermis serves to differentiate this lesion clearly from epithelioma.

Among the frequent misconceptions of Paget's disease, that of "an eczematous condition of the nipple and areola terminating in epithelioma" is about the worst. How common these misconceptions are, a glance at the text-books on surgery and pathology will show. They originate in part from association of the skin lesion with a malignant tumor of the breast and the old confusion of the terms carcinoma and epithelioma.

Whatever the genetic connection between the skin lesion and the breast tumor may prove to be, it is quite certain that in the reported cases which have been examined microscopically and in the present case, the tumor of the breast is not an epithelioma, nor have any authentic cases of an epithelioma growing from the epidermis of the nipple and areola, on the basis of a Paget's disease, or from there invading the breast, been reported. On the other hand speculation in this regard has been most industriously indulged in.

Another source of error leading to the misconception of epithelioma originating from Paget's disease, lies in the fact that carcinoma originating in the lactiferous ducts may infiltrate the corium to a considerable extent. The cells derived from the epithelium of the larger lactiferous ducts closely resemble the lower cells of the epidermis, which is not surprising when we consider the close genetic relationship

of the ducts with the epidermis of the nipple and areola. The true origin of these cells infiltrating the corium may be overlooked, and from their close resemblance to the cells of the epidermis may be ascribed to an invasion of the epidermis. With elongation of the interpapillary projections of the epidermis characteristic of Paget's disease, and with the papillæ invaded by carcinoma, the sections are often very confusing. Where the epidermis previously changed by Paget's disease or not, has ulcerated over an area of corium infiltrated with carcinoma, the condition may at first be mistaken for epithelioma originating from the epidermis. More extensive examination of such specimens, including the edge of the ulcerated area, and the region of the larger lactiferous ducts as they pass through the corium, will clear up the true origin of the growth from the lactiferous ducts.

Through the absence of carcinomatous invasion of the corium from the lactiferous ducts, the growth in the larger ducts remaining confined within the surrounding elastic tissue, the present specimen offers an excellent opportunity for obtaining clear pictures of this disease.

The term malignant papillary dermatitis was applied to this affection by George Thin, to whom we owe the first and most excellent histological study of the disease. It is quite clear from a perusal of his report that the adjective "malignant" was applied with reference to the association of this disease with carcinoma of the mamma, not with the idea that the skin affection was itself a carcinoma or became a carcinoma. On the contrary, he clearly describes the carcinoma as originating from the lactiferous ducts, and coined another term, namely, "duct carcinoma," to distinguish this type from "adenoma" and "scirrhus carcinoma."

Thin pointed out two modes of growth, namely, an increase in the thickness of the epithelial wall of the duct, the surrounding elastic tissue remaining intact, and secondly, an invasion of the stroma by cells which have broken through this tissue.

In the present specimen the growth in the larger lactiferous ducts is limited to an increase in size of the ducts, through hyperplasia of the epithelial cells of their walls. In the degeneration noted in the cells bordering the lumen, and the inflammatory reaction in the neighboring stroma, the changes bear some resemblance to those found in the skin. There is, however, a marked difference. The degeneration does not extend throughout the entire epithelial wall with complete disintegration as it does in the epidermis. Notwithstanding degeneration of the inner layer, growth and multiplication continues in the

outer. Eventually, the growth of epithelium breaks through the surrounding elastic tissue and invades the stroma.

The changes in the terminal ducts and their neighboring alveoli (Fig. 5) present the picture of adeno-carcinoma. These areas occupy a very small extent of the cross section through the breast (below and to the right in Fig. 1). Such areas, judging from other specimens of carcinoma of the breast in which all stages could be made out, eventually develop into islands of epithelial cells, all trace of lumen or of glandular structure being lost. These islands are larger or smaller, and closely adjoining one another or scattered, depending upon the accompanying proliferation of the stroma.

The appearance of carcinoma of the breast varies primarily according to the parts of the epithelial structure from which the growth takes its origin, namely, larger lactiferous ducts, terminal ducts, or alveoli; secondly, according to the advance in the growth and further departure from glandular structure in appearance; thirdly, according to presence or absence of invasion of epithelial cells in the lymph spaces of the stroma; and finally according to the extent of proliferation of the stroma. If numerous sections are made from different parts of a carcinoma of the breast, some of these different types are often found in the same tumor.

The type of carcinoma in Thin's[1] four cases, in Duhring's,[2] in Fordyce's[3] and in the present case, is chiefly "duct carcinoma." I have examined several cases of duct carcinoma of the breast in which there was no indication of Paget's disease. Although the majority of the cases are associated with carcinoma of the breast, still many of the reported cases are not.

In the present state of our knowledge the genetic connection between Paget's disease or malignant papillary dermatitis, and carcinoma of the breast, remains a matter of speculation. The two affections, although they are frequently associated, and, as pointed out, bear some points of resemblance, are nevertheless essentially different. The theory that the skin affection is caused by the irritation of carcinomatous material discharged from the ducts, fails to meet the cases in which the skin affection precedes the appearance of a tumor by many years or is not followed by a tumor at all. On the other hand, the theory that the process beginning as a surface infection, creeps down the ducts, was based on a misinterpretation of cell inclusions as para-

[1]Thin. *Brit. Med. Journal*, 1881, p. 763.
[2]Duhring. *Am. Journal. Med. Sc.*, 1884, p. 141.
[3]Fordyce. *N. Y. Med. Jour.*, Oct. 2, 1897.

sites. Moreover, the process in the epidermis and in the ducts is not the same.

From the fact that malignant papillary dermatitis is incurable, and in the majority of cases is associated with carcinoma of the breast, the extirpation of the breast with the diseased area of the skin, without waiting for the clinical diagnosis of a tumor of the breast, seems advisable.

A "duct carcinoma" may be slow in growth. Moreover, the resistance or mass felt beneath the skin upon which the diagnosis of tumor is usually made, is really due in many cases more to the dense fibrous stroma than to the actual increased amount of epithelial or carcinomatous new growth. Yet tumors of this description may, when their epithelial elements once gain access to lymphatics, produce extensive metastases, which from the clinical appearance of the primary tumor are entirely unexpected.

DESCRIPTION OF PLATES.—To Accompany Dr. Geo. T. Jackson's Case of Paget's Disease.

FIG. 1. (x 10.) Malignant papillary dermatitis and duct carcinoma of the breast. Transverse section through the skin and tumor. Above, normal skin to the left side, the area of diseased skin is on the right. The skin was somewhat folded. The area of malignant papillary dermatitis begins just to the left of the crest of the fold. The progressive thickening of the epidermis and corium from this point to the right is apparent. The tumor presents a dense stroma in which the cross section of numerous ducts are found, their epithelial walls are markedly thickened, some are empty, others contain degenerated epithelial cells. Below and to the right, areas of smaller ducts with a growth in the neighboring alveoli, are presented.

FIG. 2. (x 45.) Sections of smaller ducts with neighboring alveoli showing a growth of epithelial cells in both, which here invade the adjacent stroma. The latter shows numerous areas of infiltration with lymphocytes and plasma cells.

FIG. 3. (x 45.) Malignant papillary dermatitis. The peripheral part of the skin lesion, showing on the right side the earliest change in the deeper layer of the prickle cells. The epidermis progressively thickens and degenerates. The basal layer is sharply marked off from the corium. The papillary layer shows an increase in the size of the papillæ, which are infiltrated with lymphocytes and plasma cells. The same infiltration is seen about vessels in the deeper part of the corium.

FIG. 4. (x 45). Transverse section of a fissure. Growth and degeneration of the epidermis, is shown, on the left side quite to the basal layer. The latter is more intensely stained in parts compressed, and is clearly marked off from the infiltrated papillary layer of the corium.

FIG. 5. (x 45.) The area of ulceration. Above and to the right the basal layer of the epidermis is still sharply marked off from the corium. On the rest of the surface the corium is denuded of epidermis except a fragment of the basal layer here and there. There is a new growth of connective tissue cells and new blood vessels, intense congestion, and dense infiltration with lymphocytes, plasma cells and a few polynuclear leucocytes.

PLATE XXIII.—To Illustrate Dr. Geo. T. Jackson's Article.

Fig. 1. (X 10.)

PLATE XXIV.—To Illustrate Dr. Geo. T. Jackson's Article.

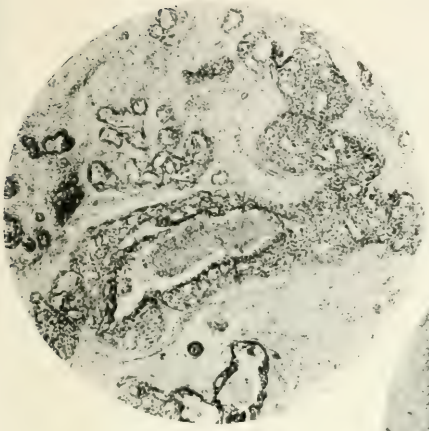

Fig. 2. (X 45.)

Fig. 4. (X 45.)

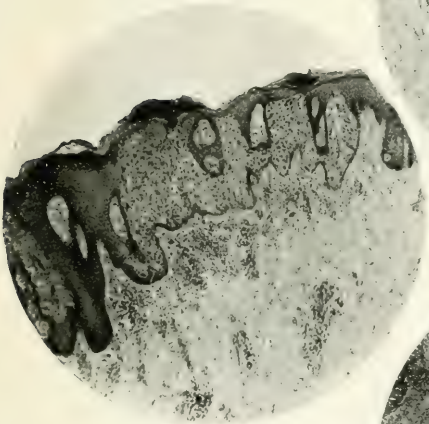

Fig. 3. (X 45.)

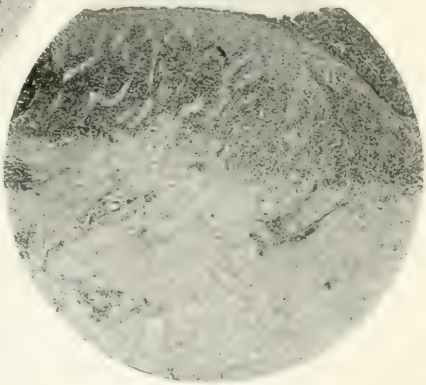

Fig. 5. (X 45.)

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